.A68 H
(1)

北海道大学
水産学部研究彙報

第6巻　第1号

BULLETIN
OF THE
FACULTY OF FISHERIES
HOKKAIDO UNIVERSITY

Vol. 6 No. 1

北海道大学水産学部
函館市
昭和30年5月

FACULTY OF FISHERIES, HOKKAIDO UNIVERSITY
HAKODATE, JAPAN
MAY, 1955

目　次

Changes in catalase activity of the tissues and blood of "Masu", *Oncorhynchus masou*, when transferred from fresh-water to sea water ····· Shinjiro KOBAYASHI (1)
Vertical distribution of mackerel eggs in Ishikari Bay, Hokkaido ····· Sigeru MOTODA (7)
Occurrence of mackerel eggs in Ishikari Bay, Hokkaido, in June, 1954, as investigated by underway plankton catcher ····· Sigeru MOTODA & Masateru ANRAKU (12)
Further observation on the daily change in amount of catches of plankton animals in vertical hauls ····· Sigeru MOTODA & Masateru ANRAKU (15)
Regional distribution of organic matter in bottom sediments in the vicinity of Okushiri Strait, Hokkaido ····· Kenji KATO (19)
海藻の窒素同化機構に関する研究-VI. 海藻の部位及び体長による硝酸還元酵素作用力の消長 ····· 高木光造・村田喜一 (25)
海藻の窒素同化機構に関する研究-VII. 硝酸還元酵素に対する阻害剤の影響 ····· 高木光造・村田喜一 (29)
海藻の窒素同化機構に関する研究-VIII. 硝酸還元酵素の中間水素伝達体に就て····· 高木光造・村田喜一 (33)
Studies on the nutritive value of the meat of sea cucumber (*Stichopus japonicus SELENKA*). V. Nitrogen distribution and amino acid composition of protein of meat of *Stichopus japonicus* ····· Eiichi TANIKAWA & Hirotoshi ISHIKO (37)
Studies on the nutritive value of the meat of sea cucumber (*Stichopus japonicus SELENKA*). VI. Nitrogen distribution and kind of chemical components of the extractives of the meat and skin parts of *Stichopus japonicus* ····· Eiichi TANIKAWA, Hirotoshi ISHIKO & Terushige MOTOHIRO (42)
Studies on the nutritive value of the meat of sea cucumber (*Stichopus japonicus SELENKA*). VII. Inorganic matter in the meat of *Stichopus japonicus* ····· Eiichi TANIKAWA & Tetsuro WAKASA (49)
Studies on post-mortem changes in the chemical constitution of the meat of sea cucumber (*Stichopus japonicus SELENKA*). I. Changes in the amounts of lactic acid and glycogen in the meat of *Stichopus japonicus* during the period of rigor mortis ····· Eiichi TANIKAWA, Minoru AKIBA. Hirotoshi ISHIKO & Setsuko YOSHITANI (52)
Studies on post-mortem changes in the chemical constitution of the meat of sea cucumber (*Stichopus japonicus SELENKA*). II. Autolysis of the meat of *Stichopus japonicus* ····· Eiichi TANIKAWA, Minoru AKIBA & Setsuko YOSHITANI (57)
Studies on post-mortem changes in the chemical constitution of the meat of sea cucumber (*Stichopus japonicus SELENKA*). III. The putrefaction of the meat of *Stichopus japonicus* ····· Eiichi TANIKAWA, Terushige MOTOHIRO & Tetsuro WAKASA (63)
Studies on post-mortem changes in the chemical constitution of the meat of sea cucumber (*Stichopus japonicus SELENKA*). IV. Determination of the freshness of the meat of *Stichopus japonicus* and the limit of the freshness for use as the raw material of canned food ····· Eiichi TANIKAWA, Terushige MOTOHIRO & Tetsuro WAKASA (73)
農林産廃液の漁網防腐剤への利用. 第1報　亜硫酸パルプ廃液及び硫酸ユコチン製造廃液の硫化処理試験 ····· 五十嵐久尚・田ノ上家久 (80)
農林産廃液の漁網防腐剤への利用. 第2報　亜硫酸パルプ廃液の硝化後硫化処理試験 ····· 五十嵐久尚・田ノ上家久 (82)

CHANGES IN CATALASE ACTIVITY OF THE TISSUES AND BLOOD OF "MASU", *ONCORHYNCHUS MASOU*, WHEN TRANSFERRED FROM FRESH-WATER TO SEA WATER

Shinjiro KOBAYASHI
Faculty of Fisheries, Hokkaido University

There have been many reports on studies of osmoregulation in salmon fry, parr and smolt.[1] As possible regulators of salt and water balance in fish, some hormones such as those of the thyroid, the gonads, the interrenals and the pituitary glands have been investigated.[2] However, it is presumable that some enzymes in tissues and blood might also be related to osmoregulation in fish. In previous papers, the author has reported that the kidney catalase activity of the salmonid fishes is much higher than that of the typical fresh-water ones,[3] and that in *Oncorhynchus masou* the catalase activity of the liver, kidney and blood of the smolt is significantly higher than that of the parr, at least, in the time of the seaward migration.[4]

The present report gives the results of an experiment performed to find whether some changes of the catalase activity of the tissues and blood in the smolts of *O. masou* occurred when they were transferred from fresh-water to sea water. As the experiment was done at the time of the seaward migration of this species, it may be said that the smolts used were a physiologically marine form.[5][6]

The author wishes to acknowledge his indebtedness to Mr. T. Kubo, Assist. Prof. of Hokkaido University for supplying the fishes used. It is a pleasure to record here a debt of gratitude to Dr. V. S. Black of the University of British Columbia for his kind sending of literature. Thanks are also due to Mr. R. Yuki for generous assistance in carrying out the experiments.

MATERIALS AND METHODS

In the present experiment were used the smolts of *O. masou* which had been reared in a fresh-water pond. The experiments were performed twice in two years. The first experiment (Exp. No. 1) was done during July 24-August 5, 1953; this period is a little past the active season of the seaward migration in the smolt of this fish. The second experiment (Exp. No. 2) was performed on June 2-3, 1954; this time just corresponds to the active season of seaward migration. In spite of that the date of Exp. 1 was later than that of Exp. 2, the fishes of the former were smaller in size than those of the latter. The body weight was 16–38g and the standard length 11.5–14.7cm in Exp. 1, and the body weight 28–53g and the standard length 13.5-16.4cm in Exp. 2.

The fishes were acclimatized in dilute sea water. The periods of such acclimatization were 26 hours in Exp. 1 and 3.5 hours in Exp. 2. The dilute sea water in Exp. 1

was about 40% sea water, and its σ_{15} was 1.009. In the case of Exp. 2, sea water was dropped slowly into fresh-water during 3.5 hours, in which water the fishes were acclimatized gradually; the final σ_{15} of this dilute sea water was 1.006. Then the fishes were transferred to sea water. The σ_{15} of sea water used was 1.024 in Exp. 1 and 1.025 in Exp. 2. In both the acclimatization and experiment, aquaria of about 21 liters in Exp. 1 and of about 29 liters in Exp. 2 were used. Into these respective aquaria were transferred 17 fishes in Exp. 1 and 31 fishes in Exp. 2; the water was constantly aerated and renewed every other day. The water temperature was varied from 14.6° to 15.6°C in Exp. 1, and from 10.5° to 11.0°C in Exp. 2. In both experiments, the fishes were fed sparingly with dried and ground crustacean larvae in the aquarium. Although the cause was not clear, the fishes of Exp. 1 fed well, while those of Exp. 2 ate little. As the control fishes, smolts were used which were collected from the pond in which they had been reared. However, examinations were made to ascertain whether or not the catalase activity in tissues and blood changes when the fishes are confined several days to a small aquarium. Table 1 shows results, however, for parrs of *O. masou*, which

Table 1. Influence on the catalase activity in the tissues and blood of the parr of *Oncorhynchus masou* when confined two weeks to a fresh-water aquarium with running water; July 29–August 12, 1953

Tissues and blood / Group	Catalase activity M±standard error of the mean*						No. of fishes examined
	Liver	Kidney	Blood	Gill	Stomach	Muscle	
Pond	76±2.7	16±1.8	19±2.1	1.6±0.35	0.55±0.164	0.66±0.017	6
Aquarium	61±2.1	13±2.2	16±0.8	2.4±0.68	0.97±0.196	0.32±0.076	5
*P***	<0.005	>0.3	>0.2	>0.3	>0.1	<0.005	

* Standard error of the mean $=\sqrt{d^2/n(n-1)}$

** Probability was calculated from the table of Student's t, using the equations:

$$t=m_1-m_2\Big/\sqrt{SE_1^2-SE_2^2} \quad \text{and} \quad n=(n_1-1)+(n_2-1),$$

where m_1 and m_2 represent the two means, SE_1 and SE_2 the two standard errors, and n_1 and n_2 are the number of fishes from which the respective means are obtained.

were confined two weeks to a fresh-water aquarium through which running water was flowing. It will be seen that the significant falls were found only in the catalase activity of the liver and the musle. Possibly, similar falls might be observed in the smolts if examined.

The methods of enzyme preparation, and of the estimation and calculation of catalase activity are the same as those of the previous experiments.[3)4)] That is, the catalase activity was calculated in k per dilution rate of tissue wet weight g, except that of the blood where it was calculated in k per dilution rate of the blood volume sampled.

RESULTS

The results obtained are given in Tables 2 and 3. The changes of the catalase activity in the liver, kidney and blood are illustrated in Figures 1 and 2.

Liver. In Exp. 1 the catalase activity increased at 6 hours in the dilute sea water to a highly significant degree ($P < 0.001$). However, in both experiments no significant changes of the catalase activity were found after transference of the fishes from the dilute sea water to sea water.

Table 2. Changes of the tissue catalase activity in the smolts of *O. masou* in dilute sea water and in sea water (Exp. No. 1); July 24–August 5, '53

Tissues / Time	Catalase activity M±standard error of the mean					No. of fishes examined
	Liver	Kidney	Gill	Stomach	Muscle	
6 hrs in dilute sea water	69±0.2	18±1.8	1.9±0.13	1.3±0.27	0.20±0.034	5
P	<0.001	——	——	>0.7	>0.2	
12 hrs in sea water	63±3.0	13±0.7	2.2±0.05	0.63±0.184	0.26±0.142	4
P	>0.1	<0.001	>0.1	<0.05	>0.6	
24 hrs in sea water	63±4.1	17±1.3	2.1±0.38	0.79±0.150	0.14±0.017	5
P	>0.2	>0.5	>0.6	>0.05	>0.1	
48 hrs in sea water	62±3.3	17±0.9	3.7±1.78	0.90±0.149	0.58±0.200	4
P	>0.3	>0.4	>0.3	>0.1	>0.3	
12 days in sea water	59±1.2	16±0.7	2.4±0.25	2.0±0.32	0.22±0.026	8
P	>0.6	>0.05	>0.1	<0.05	>0.3	
Control (Pond)	58±1.7	18±0.7	1.9±0.17	1.2±0.12	0.34±0.120	8

Table 3. Changes of the catalase activity in the tissues and blood of the smolts of *O. masou* in sea water (Exp. No. 2); June 2–3, '54

Tissues and blood / Time in sea water	Catalase activity M±standard error of the mean			No. of fishes examined
	Liver	Kidney	Blood	
3 hrs	53±7.1	9.4±1.95	15±3.0	5
P	>0.6	>0.1	>0.5	
6 hrs	53±3.8	8.5±1.63	16±2.9	5
P	>0.4	<0.05	>0.7	
12 hrs	46±3.1	11±1.7	15±0.7	5
P	>0.4	>0.3	>0.2	
18 hrs	41±3.4	12±0.5	16±1.6	6
P	>0.05	>0.3	>0.6	
Control (Pond)	49±2.8	13±0.8	17±1.6	9

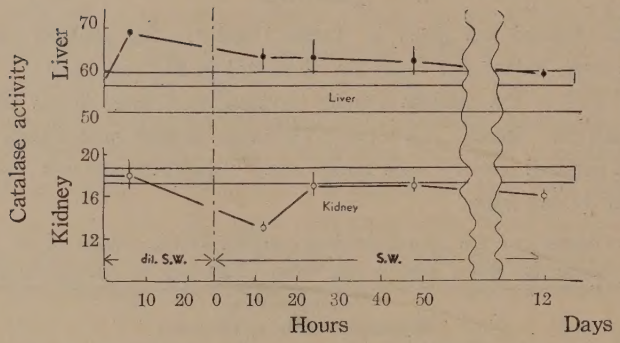


Fig. 1. Changes in the catalase activity of the liver (●) and kidney (○) in the smolts of *O. masou* in dilute sea water and in sea water (Exp. No. 1); each bar indicates the standard error of the mean in the experimental fishes and the height of each rectangle shows that in the respective control.

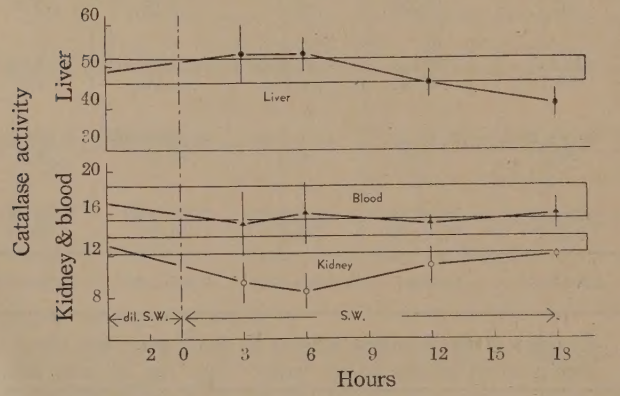


Fig. 2. Changes in the catalase activity of the liver (●), kidney (○) and blood (▲) in the smolts of *O. masou* in sea water (Exp. No. 2); each bar indicates the standard error of the mean in the experimental fishes and the height of each rectangle shows that in the respective control.

Kidney. In the dilute sea water, no significant changes were found in the catalase activity in both experiments. However, in sea water the catalase activity fell at about 6-12 hours and returned to the normal level after about 12-24 hours. The depression was highly significant ($P < 0.001$) in Exp. 1 and probably significant ($P < 0.05$) in Exp. 2.

Blood. The changes in the catalase activity of the blood were examined only in Exp. 2. No significant changes were found, at least, within 18 hours, though the individual

variations were large during the initial 6 hours in sea water.

Stomach. No significant changes of the catalase activity were found at 6 hours in the fishes in the dilute sea water. However, the enzymatic activity decreased at 12 hours in the fishes in sea water, and the depression was probably significant ($P < 0.05$). Even after 24 hours in sea water, the catalase activity appeared to retain the low level, though the fall in this period was probably non-significant ($P > 0.05$). After 48 hours in sea water the catalase activity appeared, at first, to approach the normal level. However, it increased conversely after 12 days in sea water, and the increase was probably significant ($P < 0.05$).

Gill and muscle. In tissues of both gill and muscle, no significant changes of the catalase activity were found either in the fishes kept in sea water or in those in dilute sea water.

DISCUSSION

It has been reported that Atlantic salmon smolts can survive direct transfer to sea water.[1,7,8] Similarly, the smolts of *O. masou* could be transferred from fresh-water to sea water directly. However, a method of acclimatization in the dilute sea water was adopted taking the natural migration of the fish into consideration, and as the time for that acclimatization 3.5 and 26 hours were selected in the present experiments.

As described in the previous papers,[3,4] the physiological significance of the catalase activity in the tissues and blood is not clear at present. However, it is noticeable that the kidney catalase activity depressed at about 6-12 hours and returned to the normal level after about 12–24 hours when the smolts were transferred from fresh-water to sea water; these times just agree with those of both the marked increase and return to the normal level in the body chloride content of the chum salmon fry in sea water.[9] Further, in this connection it is very suggestive that changes of the structure of the tubules, unusual increase of the secretion in them, and agglomerated appearance of melanin in the connective tissue cells have been observed in the kidney of goldfishes which were reared several days in dilute sea water.[10]

No significant changes were found in the blood catalase activity. That may be ascribed to the evidence that this enzyme exists mainly within the blood cells being protected by their membranes.[11,12] However, as is clear judging from the values of the standard error of the mean, the standard deviations of the blood catalase activity are considerably large, at least, at 3–6 hours in sea water, though the changes were not significant. Similarly large fluctuations of the enzymatic activity could also be found in such tissues as those of liver and kidney in the initial several hours in sea water.

The depression of the stomach catalase activity may be associated with the fact that the fish drinks sea water. Contrary to expectation[1,9,13] no significant changes were found in tissues either of the gill or of muscle. It is not clear at present whether that is

actually the case or whether small changes but large relatively to the very low levels of these tissues may occur which could not be estimated by the present method.

SUMMARY

The smolts of *Oncorhynchus masou* were transferred from fresh-water to sea water after being acclimatized in dilute sea water. On these fishes, the changes of the catalase activity in the tissues and blood were examined at various time intervals.

1. The liver catalase activity showed a significant change at 6 hours after transference of the fishes to the dilute sea water. However, no significant changes were found in transferences to sea water.

2. In sea water, the kidney catalase activity of the experimental fishes decreased significantly at about 6–12 hours and returned to the normal level at about 12-24 hours.

3. Any significant changes of the blood catalase activity were not found. However, a large fluctuation of the enzymatic activity was observed at the initial 3–6 hours in sea water.

4. Significant depression of the stomach catalase activity was found at 12 hours in the experimental fishes in sea water.

5. In tissues of both the gill and muscle, no significant changes were found.

LITERATURE CITED

1) Black, V. S. (1951). Osmotic regulation in teleost fishes. *Toronto Stud. Biol.* **59**; *Pub. Ontario Fish. Res. Lab.* **71**, 53-89.

2) Hoar, W. S. (1951). Hormones in fish. *Ibid.* 1-51.

3) Kobayashi, S. & Yuki, R. (1954). On the specificity of kidney catalase activity in salmonid fishes. *Bull. Fac. Fish., Hokkaido Univ.* **5**, 137-148.

4) ——— & ——— (1954). Differences in catalase activity in the tissues and blood between the smolt and parr of Masu, *Oncorhynchus masou*. *Ibid.* **5**, 223-230.

5) Hoar, W. S. (1953). Control and timing of fish migration. *Biol. Rev.* **28**, 437-452.

6) Kubo, T. (1954). Some nitrogen compounds of blood and metamorphosis of *Oncorhynchus masou*. *Bull. Fac. Fish., Hokkaido Univ.* **5**. 248-252. (in Japanese).

7) Huntsman, A. G. & Hoar, W. S. (1939). Resistance of Atlantic salmon to sea water. *J. Fish. Res. Bd. Canada* **4**, 409-411.

8) Vibert, R. (1948). Travaux effectués en France sur le saumon. *J. Conseil* **15**, 328-333.

9) Black, V. S. (1951). Changes in body chloride, density, and water content of chum (*Oncorhynchus keta*) and coho (*O. kisutch*) salmon fry when transferred from fresh water to sea water. *J. Fish. Res. Bd. Canada* **3**, 164-177.

10) Kato, K. (1951). [Influence of various dilutions of sea water on fish.] *Zool. Mag.* **60**, 28. (in Japanese; abstract of the Annual Meeting of the Zool. Soc. Japan).

11) Sekiguchi, A. (1953). Studies on the catalase activity of red cell suspension. 1. Red blood cell suspension as an enzyme solution. *J. Physiol. Soc. Japan* **15**, 357-367. (in Japanese).

12) Yamagata, S. & Nakao, T. (1952). Studies on blood and plasma catalase. II. The blood catalase content in digestive disease patients. *Tohoku J. Exp. Med.* **57**, 93-99.

13) Nishihara, H. (1953). On the changes of chlorine content of goldfish in sea water. *Sci. Rept. Saitama Univ.*, Ser. B., **1**, 71-75.

VERTICAL DISTRIBUTION OF MACKEREL EGGS IN ISHIKARI BAY, HOKKAIDO*

Sigeru MOTODA
Faculty of Fisheries, Hokkaido University

For determining the vertical distribution of eggs of mackerel (*Scomber japonicus HOUTTUYN*) at their spawning ground off Hamamasu, west coast of Hokkaido, serial horizontal tows with six nets were made at every successive four hours on 23rd and 24th, June, 1954. The net tows were made usually for an hour by the movement of ship drifting in moderate wind. During tows the angle of warp carrying the nets against the vertical line measured approximately 45 degrees, so that the towing strata of six nets were calculated to correspond to about 0.7 m, 1.1 m, 4.2 m, 7.7 m, 11.2 m and 14.7 m depth respectively, though these depths may have fluctuated to some extent by the condition of drift of the ship. In these tows a flow meter developed by Mr. Z. Nakai was held rigidly at the centre of the mouth ring of the second net at 1.1 m depth to register the distance of tows. The revolution of flow meter has been calibrated to be 153 in mean from four experiments for 20 – 0 metre vertical haul in which the net had been removed from the ring, so that 100 revolution was calculated to be corresponding to the distance of tow as long as 131 metres when there was not any resistance due to anything such as the net.

The nets employed in collecting the eggs were constructed of No. 40 grit gauze having approximately 0.6 mm of opening between meshes. They were conical in shape, 35 cm in diameter at the mouth (about 0.1 m^2 in mouth area) by approximately 165 cm in length. Thus, the 100 revolution of flow meter attached to the present nets indicates that water to the amount of about 12.6 m^3 has been filtered. Vertical hauls from 20 m, 15 m, 10 m and 5 m depth to the surface with the same net as above, yielded only occasional eggs, only one egg if present for a haul, so that the contamination of samples from overlying layers which may have occurred when the nets were lowered and retrieved in the serial horizontal tows was considered to be negligible, needless to adjust for the samples obtained by horizontal tows.

Among the pelagic eggs sampled those of which the diameter was one mm or thereabout and in which an oil globule was contained were tentatively counted as mackerel eggs. Although exact identification of pelagic eggs was impossible on such materials as preserved in formalin, the majority of the eggs counted as mackerel's were thought to be the eggs of this fish because of the presence of a large shoal of spawning adults in the area which presupposed the occurrence of their eggs in the sea.

---

* 水産庁委託対馬暖流調査報告 No. 2

There were seen various stages of development among the samples which were treated as mackerel eggs, but separation of stages in counting the number of occurrence was made only into two groups for convenience, viz., the eggs in early stage, i. e. still in blastodisc stage (stage I) and the eggs in later stage including various developmental grades of embryonic stage (stage II) (fig. 1). The eggs in stage II were supposed to have passed at least more than 20 hours after spawning at the temperature of the sea surface in the present area, about 13°C (cf. Kamiya, 1916, 1925).

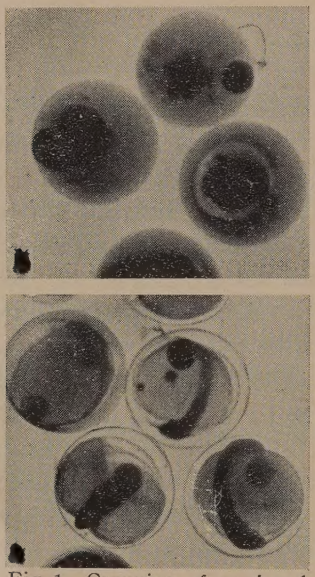


Fig. 1. Grouping of mackerel eggs into two kinds
a. Stage I b. Stage II

Table 1 gives the number of two stages of mackerel eggs together with the displacement volume of total plankton collected at different strata and at different times of a day; the simplified figures illustrating the occurrence of mackerel eggs are given in figure 2. In this table and figure the number of eggs and the volume of total plankton are expressed as the number or volume in 100 m^3 of water by adjusting for readings of flow meter at each tow. Although irregularities of the data cause confusion in finding general tendency of distribution of the eggs, certain mention will be made comparing with the results of previous investigators.

It has widely been believed that mackerels spawn at night as proved in the case of the California sardines and the Japanese sardines. Kamiya (1916, 1925) was of the opinion that mackerels spawn during the time mainly from sunset to midnight. According to the observations of Sette (1943) on the Atlantic mackerels, however, the proportion of number of occurrence of two stages of eggs, one in the stages up 10 hours after spawning and another in the stages up to 20 hours, in nature was not apparently different by the times of a day. In the present collections the eggs in stage I, as well as those in stage II to some extent, are comparatively abundant at about 5:00 p. m., but whether major spawning has taken place a certain number of hours before this time cannot yet be concluded from this single datum. It is more probable that the eggs in concentration may have been drifted to this area by current at that time. The results are not sufficient for determining the spawning time, unless more precise separation of the stages in young eggs is made and more precaution for sampling in keeping the same water during 24 hours is taken.

Sette (1943) found that the mackerel eggs in more advanced developmental stage, were distributed deeper. In the present observation the eggs in later stage were rare

Table 1. Results of serial horizontal tows

Time of sampling	Amount of water filtered by the net	Depth of sampling	Displacement of total plankton in 100 m^3 of water	Number of mackerel eggs in 100 m^3 of water	
				Stage I	Stage II
23, June		0.7 m	<1 cc	2	0
		1.1 m	<1 cc	0	0
1 : 22 p.m.	57m^3	4.2 m	<1 cc	5	0
-2: 22 p.m.		7.7 m	<1 cc	4	5
		11.2 m	<1 cc	9	9
		14.7 m	<1 cc	18	5
		0.7 m	<1 cc	140	9
		1.1 m	<1 cc	55	0
4 : 34 p.m.	22m^3	4.2 m	<1 cc	9	0
-5: 34 p.m.		7.7 m	<1 cc	100	55
		11.2 m	1.9 cc	46	36
		14.7 m	<1 cc	23	9
		0.7 m	6.4 cc	10	4
		1.1 m	4.4 cc	4	2
8 : 35 p.m.	52m^3	4.2 m	9.6 cc	19	15
-9: 35 p.m.		7.7 m	13.5 cc	63	56
		11.2 m	7.9 cc	27	19
		14.7 m	7.6 cc	100	21
24, June		0.7 m	8.9 cc	8	3
		1.1 m	9.0 cc	3	3
1 : 05 a.m.	71m^3	4.2 m	9.9 cc	7	0
-2: 36 a.m.		7.7 m	11.5 cc	34	1
		11.2 m	13.6 cc	21	1
		14.7 m	12.4 cc	14	0
		0.7 m	<1 cc	2	0
		1.1 m	<1 cc	2	3
4 : 35 a.m.	119m^3	4.2 m	<1 cc	3	4
-5: 35 a.m.		7.7 m	2.3 cc	16	6
		11.2 m	<1 cc	3	0
		14.7 m	1.9 cc	2	1
		0.7 m	<1 cc	0	0
		1.1 m	<1 cc	0	0
8 : 30 a.m.	56m^3	4.2 m	1.8 cc	0	7
-9: 30 a.m.		7.7 m	<1 cc	2	11
		11.2 m	<1 cc	12	14
		14.7 m	1.4 cc	11	14

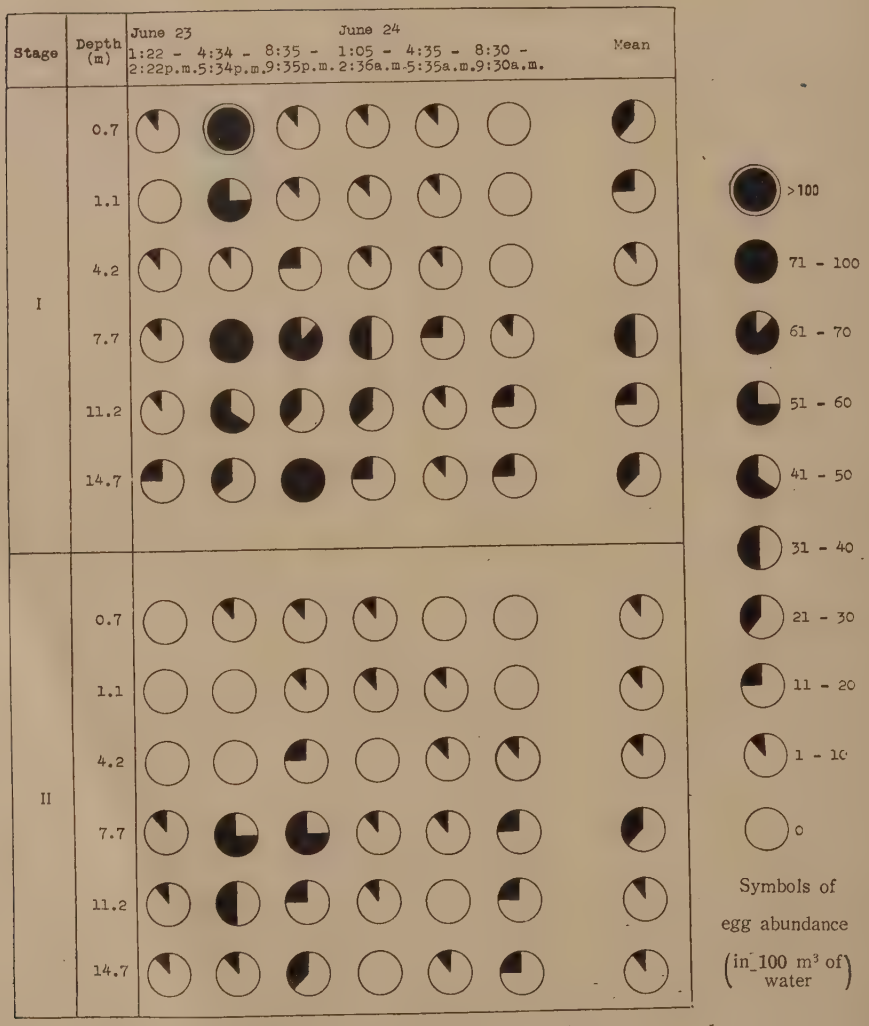


Fig. 2. Occurrence of mackerel eggs in various depths at various times of a day

Table 2. Gradient of temperature

Depth (m)	Temp. (°C)
0	12.95
5	12.80
10	12.50
15	12.20
20	11.10
25	10.20
30	9.90
35	9.60

above 4 m depth as compared with those at about 8 m depth, while the number of eggs in early stage was not reduced in the upper layers. The observation of Sette (1943) proved that the mackerel eggs as a whole were distributed in large number in upper 10 metres, but nearly absent at 20 m layer. In his case the lower limit of distribution of the eggs was disturbed by the existence of discontinuity of density of water at 10—20 m depth. In the present observation temperature was nearly uniform from the surface to about 15 m depth (table 2), and the eggs as a whole were sometimes found in considerable number through the vertical range from the surface to at least about 15 m depth, below which presence or absence of eggs was unascertained owing to the lack of collection.

The author is much indebted to Mr. N. Hanamura, Mr. I. Takeuchi, Mr. M. Anraku and Mr. O. Sano for their kind help during the present observation.

References

Kamiya, T. (1916). The pelagic eggs and larvae of fishes in the Tateyama Bay, (Pref. Chiba). *Jour. Imp. Fish. Inst.* **11** (5), 37-42. (in Japanese).

——— (1925). The pelagic eggs and larvae of fishes on the coast of "Hokuriku" (Northwestern part of Japan Proper). *Ibid.* **21** (3), 86-106. (in Japanese with English summary).

Sette, Oscar Elton (1943). Biology of the Atlantic mackerel (*Scomber scombrus*) of North America. Part I. Early life history including the growth, drift and mortality of the egg and larval populations. *U. S. Fish and Wildlife Service* **50**, *Fishery Bulletin* 38, 147—237.

OCCURRENCE OF MACKEREL EGGS IN ISHIKARI BAY, HOKKAIDO, IN JUNE, 1954, AS INVESTIGATED BY UNDERWAY PLANKTON CATCHER *

Sigeru MOTODA and Masateru ANRAKU
Faculty of Fisheries, Hokkaido University

Mackerel fishery is commonly carried on from June to July in the Ishikari Bay and the adjacent area off the west coast of Hokkaido when mackerels (*Scomber japonicus HOUTTUYN*) are concentrated there to spawn. In order to figure the distribution of mackerel eggs in this region the present brief research was undertaken in late June, 1954. The underway plankton catcher, model II, (Motoda, 1954) was towed from fishing boat while underway at about 9 knots for convenient duration ranging from 13 minutes to one hour along the course between each pair of hydrographic stations. It is proved from this brief trial tow that this method is useful for determining the distribution of the pelagic eggs in the surface water.

Counting the number of mackerel eggs was done by classifying them into two groups, viz., the eggs in blastodisc stage and those in more advanced stage (Motoda, 1955). Table 1 and figures 1 and 2 give the occurrence of those two stages of mackerel eggs in the collections. In the table and figures the number of occurrences was in all cases converted to the number in 100 cubic metres of water. The underway catcher used can filter 100 cubic metres of water during a tow of three hours and a half, assuming that the water passing through the mouth opening of catcher has completely filtered through the net inside.

It is shown from the figures that the eggs of early stage were abundant along the Takashima-Hamamasu line, particularly concentrated near Takashima, while the eggs of more advanced stage were present in considerable number off the northeast coast of the Shakotan Peninsula, and in small number along the Takashima-Hamamasu line. This indicates that, if there was no drift of the eggs by currents during the period of the present observations, the spawning of mackerels must have early taken place off the Shakotan Peninsula, and later near the Takashima and on the eastern coast of the bay. No eggs at all were found in the offing of the central region of the bay where the water was comparatively warmed up.

Takeuchi (1955) has counted the mackerel eggs in the samples taken by one metre fry net towed at the surface during the same cruise as the present sampling. He got less than 17 mackerel eggs per about 100 m^3 of water at the most at any one station, and even if the allied eggs were counted together, the largest number at anyone station was about 30. The present data show comparatively high value as compared with those

* 水産庁委託対馬暖流調査報告 No. 3

data obtained by him.

The authors are much indebted to the Fisheries Agency for making possible the present work. They also wish to express deep thanks to Mr. N. Hanamura of the Hokkaido Regional Fisheries Research Laboratory for his kindness in providing facilities during the work, and to Mr. I. Takeuchi and Mr. O. Sano of the same Laboratory as well as to Mr. S. Nishizawa and Mr. M. Fukuda of the University for help in sampling. Thanks are also due to Prof. N. Inoue of the University and his colleagues for providing the hydrographic data.

References

Motoda, S. (1954). Handy underway plankton catchers. *Bull. Fac. Fish., Hokkaido Univ.* **5** (2), 149-152.

———— (1955). Vertical distribution of mackerel eggs in Ishikari Bay, Hokkaido. *Ibid.* **6** (1), 7-11.

Takeuchi, I. (1955). [Investigations on pelagic fish eggs and larvae] (1954). No. 4, 5. Hokkaido Reg. Fish. Res. Lab. (Mimeogr.) (in Japanese).

Table 1. Results of collections of mackerel eggs by underway catcher in Ishikari Bay in June, 1954

Date	Time	Range	Number of eggs in 100 m³ of water	
			Stage I	Stage II
20 June 1954	1 : 00 p.m.- 1 : 30 p.m.	Takashima-St. 1	246	28
21 June 1954	7 : 35 a.m.- 8 : 15 a.m.	Takashima-St. 1	127	102
〃	9 : 00 a.m.- 9 : 25 a.m.	St. 1 — St. 2	25	145
〃	10 : 20 a.m.-10 : 40 a.m.	St. 2 — St. 3	11	158
〃	11 : 50 a.m.-12 : 10 p.m.	St. 3 — St. 4	0	11
〃	12 : 57 p.m.- 1 : 15 p.m.	St. 4 — St. 5	0	0
〃	2 : 25 p.m.- 3 : 05 p.m.	St. 5 — St. 6	7	39
〃	4 : 00 p.m.- 4 : 35 p.m.	St. 6 — St. 7	0	0
〃	5 : 33 p.m.- 6 : 10 p.m.	St. 7 — St. 8	0	0
〃	7 : 05 p.m.- 7 : 33 p.m.	St. 8 — St. 9	0	0
〃	8 : 25 p.m.- 8 : 47 p.m.	St. 9 — St.10	0	0
〃	9 : 40 p.m.-10 : 05 p.m.	St.10 — St.11	7	0
〃	10 : 53 p.m.-11 : 30 p.m.	St.11 — St.12	0	0
22 June 1954	0 : 15 a.m.- 0 : 50 a.m.	St.12 — St.13	32	0
〃	7 : 25 a.m.- 7 : 46 a.m.	Hamamasu-St.14	21	0
〃	8 : 27 a.m.- 8 : 59 a.m.	St.14 — St.15	60	0
〃	9 : 42 a.m.-10 : 00 a.m.	St.15 — St.16	0	0
〃	11 : 03 a.m.-11 : 16 a.m.	St.16 — St.17	0	0
〃	12 : 03 p.m.-12 : 30 p.m.	St.17 — St.18	7	0
〃	1 : 18 p.m.- 1 : 43 p.m.	St.18 — St.19	42	0
23 June 1954	9 : 28 a.m.- 9 : 58 a.m.	Takashima-St.20	507	56
〃	10 : 03 a.m.-10 : 33 a.m.	St.20 — St.21	218	35
〃	10 : 45 a.m.-11 : 45 a.m.	St.21 — St.22	81	21
〃	12 : 00 m.-12 : 30 p.m.	St.22 — St.23	0	7
24 June 1954	9 : 30 a.m.-10 : 00 a.m.	St.23 — St.24	39	0
〃	10 : 10 a.m.-10 : 40 a.m.	St.24 — St.25	113	14
〃	10 : 45 a.m.-11 : 15 a.m.	St.25 — St.26	267	56
〃	11 : 20 a.m.-11 : 50 a.m.	St.26-Takashima	134	35

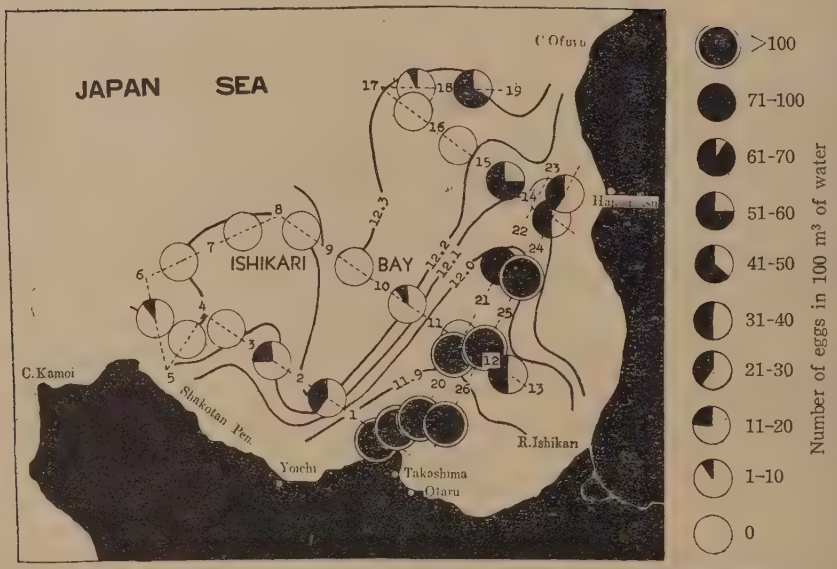


Fig. 1. Occurrence of mackerel eggs of early stage in Ishikari Bay in June, 1954. The number between the dashed lines indicates the hydrographic station and the black space in circle indicates the abundance of the eggs according to the rank given in the right. The isotherms (10 m layer) were provided by Prof. Inoue.

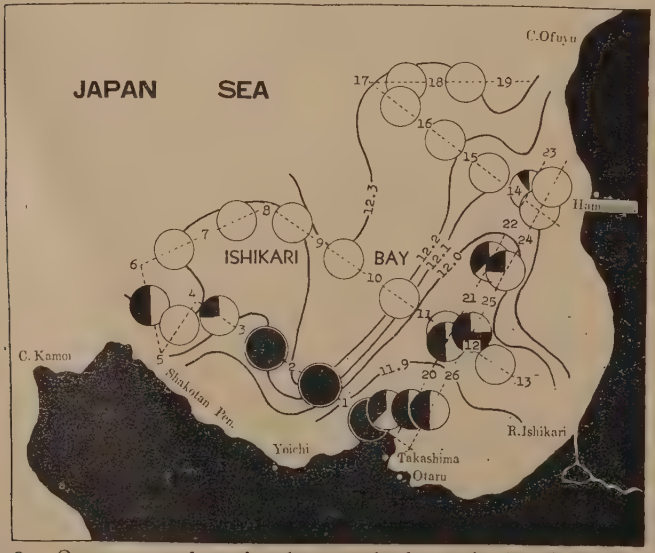


Fig. 2. Occurrence of mackerel eggs of advanced stage in Ishikari Bay in June, 1954. See the remarks in fig. 1.

FURTHER OBSERVATION ON THE DAILY CHANGE IN AMOUNT OF CATCHES OF PLANKTON ANIMALS IN VERTICAL HAULS*

Sigeru MOTODA and Masateru ANRAKU
Faculty of Fisheries, Hokkaido University

The previous observations (Motoda & Anraku, 1954) made near western entrance to the Tsugaru Strait in October, 1953, have shown that the total number of plankton animals occurring in the layers of 150–0 m, 300–0 m and 450–0 m are different in daytime from at night as expressed by the ratios 100 : 140, 100 : 141 and 100 : 126 respectively. More extensive data being required for determining the depth from which the catches by vertical hauls would yield the same amount quite irrespective of the time of day, the herein described observation was undertaken along the same line as the previous studies.

The locality of the present sampling was the offing to the eastward of Cape Esan, south coast of Hokkaido, exactly 41° 45′N, 141° 39.8′E, but the ship was shifted about around this position by one and half miles at the most during her drift for sampling. The vertical hauls from 150 m, 300 m, 450 m and 600 m depth to the surface, each repeated once, were made both in daytime (8 : 40 a. m.–11 : 08 a. m.) and at night (10 : 45 p. m.–1 : 20 a. m.) on 17th and 18th, May, 1954. The velocity of the haul was held as constant as possible at 100 cm per second. The angle of wire carrying the net sometimes attained to about 30 degrees against vertical line, but in most cases it was less than this and often the wire was suspended perfectly vertical. The wire, however, having been let out without adjustment for obtaining accurately the desired depth, the sampling depth presented here only means the length of the wire itself.

The net employed was 45 cm in mouth diameter, approximately 165 cm in length, of which the body was constructed of Japanese grit gauze No. 56, having 0.3–0.32 mm of approximate mesh aperture. The net was provided with a flow meter held rigidly at the centre of the mouth ring to register the amount of water filtered. Before the sampling, calibration of flow meter was performed by vertical hauls for 150–0 m with the flow meter kept at the centre of the ring from which the body of net was previously removed. By three experiments the revolutions were counted as 1177 in the mean, so that, when the flow meter is

Table 1. Vertical gradient of temperature and chlorinity

Depth (m)	T (°C)	Cl ‰*
0	9.90	18.79
10	10.23	18.69
25	9.36	18.74
50	9.29	18.76
75	9.61	18.74
100	8.48	18.99
150	6.49	18.76
200	3.80	18.69
300	2.53	18.73
400	2.75	18.74
600	2.99	18.85

*Thanks are due to Asst. Prof. H. Koto for titration of chlorinity.

* 水産庁委託対馬暖流調査報告 No. 4

attached on the net, 100 revolutions would correspond to 2.03 cubic metres of water presumably filtered by the net.

The gradient of temperature increased to some extent at the depth between 150 and 200 metres, though the chlorinity was kept nearly uniform through whole vertical range observed (table 1). The plankton samples consisted of a large bulk of copepods, euphausiids, mysids, amphipods, chaetognaths and hydromedusae, the remainder being a small number of ostracods, pteropods, appendicularians and polychaetes. Full data are given in table 2. It was indicated that the number of small calanids, such as *Paracalanus* and *Clausocalanus*, fluctuated irregularly and widely in repeated hauls (line 8 in table 2).

Table 2. Individual number of each plankton group found in each sample

Sampling time	Daytime								Night							
Depth (m)	150~0		300~0		450~0		600~0		150~0		300~0		450~0		600~0	
No. of sampling	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2
Calanus plumchrus	200	225	360	305	522	480	1700	1630	200	260	256	240	400	380	1920	1985
C. cristatus	0	2	22	33	34	37	46	48	3	1	23	16	24	34	76	56
Eucalanus spp.	1	2	21	35	200	208	440	548	0	0	64	56	328	280	480	444
Metridia lucens	350	342	1600	880	1600	1490	2180	2400	840	520	560	1180	1040	1920	3680	2600
Small calanids	11520	10880	12480	4480	3650	1980	11840	9600	14720	20480	8640	11840	8320	8160	10200	5160
Euphausiacea	12	23	78	57	78	87	108	80	76	109	25	62	62	71	76	47
Themisto sp.	11	8	53	27	20	29	51	33	31	17	33	23	40	42	48	32
Conchoecia sp.	0	0	32	32	40	120	195	224	0	1	16	64	130	128	128	130
Sagitta spp.	35	49	211	172	228	251	329	383	58	63	120	160	221	165	340	316
Hydromedusae	34	71	109	121	69	109	178	108	41	36	44	55	78	81	65	76
Limacina sp.	4	12	44	8	8	62	16	128	16	40	4	16	64	12	32	32
Oikopleura spp.	4	4	4	12	4	5	1	2	0	1	16	4	0	8	4	0
Polychaetes	0	0	0	0	0	2	15	5	0	0	1	1	1	3	6	12

Table 3. Total number of plankton animals excluding small calanids, *Paracalanus* and *Clausocalanus*, found in various hauls

Sampling layer (m)	Amount of water in column for 45 cm-net (m^3)	Sampling time	No. of sampling	Reading of flow meter	Amount of water filtered by the net (m^3)	Total number of plankton animals excluding small calanids					
						Number of animals actually collected	Number of animals in water column corresponding to the sampling layer	Mean for repeated samplings	Mean for day and night	Ratio of number in daytime to at night	Percentage for day-night mean
150~0	23.9	Daytime	1	870	17.7	651	879	927	1482	100	63
			2	890	18.1	738	974				
		Night	3	870	17.7	1445	1951	2036		220	137
			4	860	17.5	1548	2121				
300~0	47.8	Daytime	5	1690	34.3	2534	4282	3495	3036	100	115
			6	1774	36.0	1682	2708				
		Night	7	1685	34.2	1172	1981	2577		74	85
			8	1686	34.2	1877	3172				
450~0	71.7	Daytime	9	2510	51.0	2803	3952	3921	2824	100	103
			10	2620	53.2	2881	3889				
		Night	11	2515	51.1	2368	3315	3726		95	97
			12	2620	53.2	3064	4136				
600~0	95.6	Daytime	13	3550	72.1	4259	5664	6856	7327	100	94
			14	3279	66.6	5589	8048				
		Night	15	3830	77.8	6855	8431	7797		114	106
			16	3780	76.7	5730	7162				

This irregularity of occurrence was possibly due to inhomogenous distribution of these small calanid copepods within a small area of the sea. They might be concentrated here and there for some certain unknown reason. Therefore, to estimate the regular daily change of catches of general plankton animals, the number of plankton animals excluding the above small copepods was taken into consideration (table 3).

Table 3 indicates that the catch by haul from 150 m depth at night is more than double the catch in daytime, but the difference in catches between day and night is reduced when the haul is made from 300 m depth or below. The percentage standard deviation of haul to haul variation in vertical haul from 150m depth with 45 cm-net was calculated in an experiment about 18.6 % (about 70% and 141% of 95% fiducial limits) (Motoda & Anraku, unpublished). Applying this, the variation in catches by day and night in the present observation is significantly different only for the 150-0 m haul. The later experiments gave data on haul to haul variation (percentage standard deviation) of catches by vertical haul from about 150 m depth with such net as used in the present sampling from which was calculated a value of about 13% (about 78% and 128% of 95% fiducial limits). If the above results is applied to the present collections there occur in the 150-0 m haul significant difference in variability of catches between day and night. However, in the third series of operations carried on under the identical conditions the haul to haul variation was as large as 48%; then the 95% fiducial limits were roughly 45% and 202%, so that one count could not be considered significantly different from another one unless it was less than one-half or more than two times that value. If the last value of percentage standard deviation is applied to the present catches they are not significantly different as between day and night even in the 0-150 m layer.

King and Hida (1954) made an observation on the variability in zooplankton abundance in Hawaiian waters, obtaining an obvious day-night difference among the 200 m oblique hauls. They advocated that the collections at any time of the day would be corrected by the curve of the sine function obtained from diurnal change in catches of above levels.

The writers wish to express their appreciation to Assistant Professor T. Kawamura and fellow staff members of this University and the officers and crew of T. S. Oshoro Maru for their cooperation in obtaining the plankton samples on which this report is based. Financial aid granted from the Fisheries Agency of the Ministry of Agriculture for the present investigation is gratefully acknowledged.

Literature cited

King, J. E. & Hida, T. S. (1954). Variations in zooplankton abundance in Hawaiian waters, 1950-1952. *U. S. Fish & Wildlife Serv., Sp. Sci. Rep., Fish.* No. 118, 1-66.

Motoda, S. & Anraku, M. (1954). Daily change of vertical distribution of plankton animals near western entrance to the Tsugaru Strait, northern Japan. *Bull. Fac. Fish., Hokkaido Univ.* **5** (1), 15-19.

REGIONAL DISTRIBUTION OF ORGANIC MATTER IN BOTTOM SEDIMENTS IN THE VICINITY OF OKUSHIRI STRAIT, HOKKAIDO

Kenji KATO

Faculty of Fisheries, Hokkaido University

Introduction

As yet few available data have been published regarding regional distribution of organic substances accumulated in the bottom sediments in the seas adjacent to Japan. The author has hitherto carried out a series of investigations on areal distribution of marine humus (Kato, 1949–1955). Likewise, the Hokkaido Regional Fisheries Research Laboratory attempted during 1948–1952 to investigate submarine topography and bottom characters of the off-shore fishing grounds, at about 100–1,000 meters depth, around the coast of Hokkaido. During 23-31 of August, 1952, the area in the vicinity of Okushri Strait was investigated as one of the series of surveys by the Laboratory. As a part of that survey, the author has undertaken chemical investigation dealing with the bottom sediments, of which samples were collected by Mr. H. Kosugi of the Laboratory and Mr. S. Tanaka, a research student of the University on board "**Tankai-Maru**" (68 tons), the research vessel of the Laboratory. The survey was conducted at intervals of three miles, so the bottom characters were observed at 83 stations; 44 bottom samples were subjected to laboratory investigation involving microscopic observation and chemical or mechanical analysis (Fig.1).

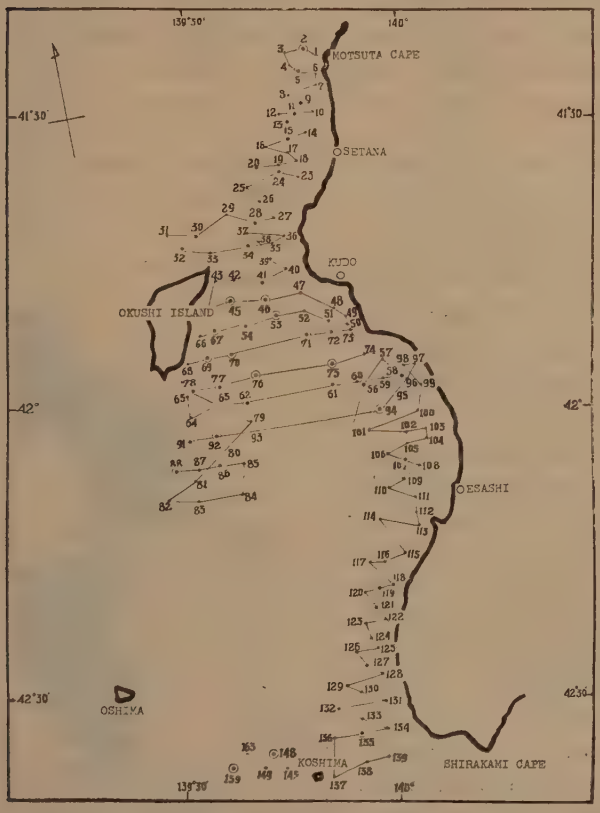


Fig. 1. Chart of observation stations

Methods employed

The oceanographic observation was carried out with good position control. For obtaining locations accurately, not only was the visible or sextant method employed, but other kinds of instrumental controls were used. Echo-sounding played in practice a very important role carrying on the investigation of topography.

Bottom samples were obtained by using a small coring device or a snapper. The samples obtained thus were submitted to laboratory investigation. To prevent deterioration, however, the larger organic remains, as benthos or weeds, found in sediments were stored in vials containing formalin solution or spirit for biological examination.

Of the bottom sample dried at 100–105°C, the organic carbon content was determined by means of the same titration method as employed by Waksman (1933), and the total nitrogen content by using the common Kjeldahl method.

As to mechanical analysis of the sample, the wet seiving method was employed for separating the fractions larger than 0.06mm in diameter, while the settling methtod was used for the finer fractions. From the results of the analysis, a cumulative frequency curve was drawn with respect to the grain size of the sample; thus the mass properties of the sample were examined.

Laboratory work, next, led to microscopic observation of planktonic remains accumulated in the sediment, for ascertainment of how the organic remains had affected the organic accumulation in the bottom floor.

Geographic view of the area and bottom character

The area under discussion is located intermediate between Motta Cape (42° 316′N) and Koshima Island (41° 19′N) neighboring the western entrance of Tsugaru Strait. Tsushima Current flows northward through the area and used to branch off forward Okushiri Island.

The character of submarine topography in the area may be emphasized as follows (Fig. 2, Ogaki *et al*, 1953): The continental shelf is very narrow, namely, about one to five miles wide, and goes down in a steep slope to the deep bottom of the Japan Sea. The channel of Okushiri lying between Okushiri Island and the Oshima Peninsula, is comparatively shallow in depth. Midway in the channel, runs a narrow trough having about 500 meters depth. A shelf lies in the vicinity of Shirakami Cape and Koshima Island. It is another characteristic of the area that a number of small submarine valleys traverse the shelf of the peninsula.

Fig. 2. Submarine topography (m)

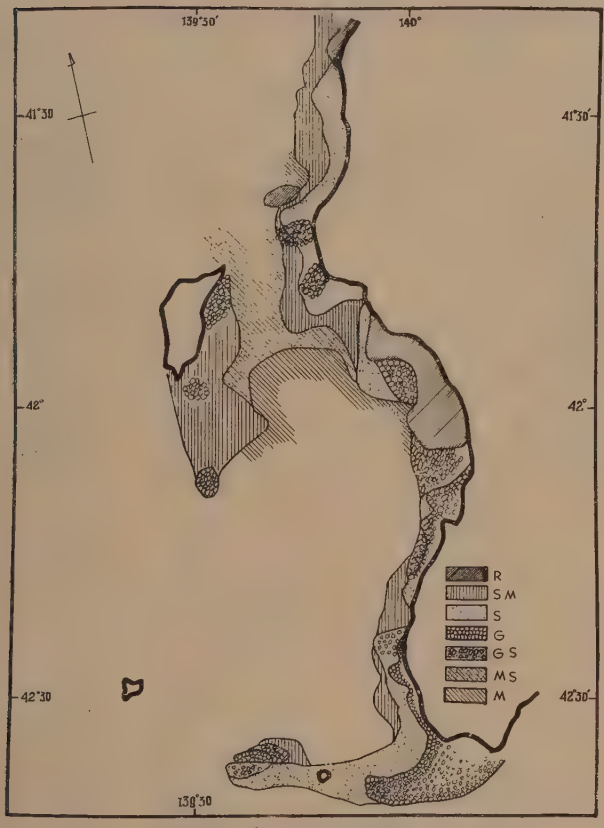


Fig. 3. Bottom character

As illustrated in Fig. 3, bottom character of the shelf is rocky, sandy or gravelly, that of the steep slope is muddy.

Results and discussion

General properties of the bottom samples from the area are summarized in Table 1, and regional distribution of organic substances in sediments is illustrated in Figs. 4-6. Some statistical examinations have been made regarding organic contents in sediments as well as their relations to the water depth of the site of deposition; the results are summarized in Table 2.

Sedimentary accumulation of organic matter in bottom floor was fairly abundant in the area; the mean contents of organic matter were indicated as 1.22% C or 0.13% N. Such abundant accumulation of organic matter appears to be one of characteristics of organic accumulation in the Japan Sea (Kato, 1951). These mean organic contents are, however, rather less abundant than those of sediments in the vicinity of Musashi Bank (1.87% C and 0.21% N,) (Kato, 1951). The amount of organic matter accumulated in sediments appears to have some relatiom to the water depth at the site of deposition, that is, the contour of organic content as shown in Figs. 4 and 5 may probably be parallel to the submarine contour of Fig. 2. So, some statistical observations were undertaken regarding the correlationship of organic accumulation to the water depth as tabulated in Table 2.

According to Table 2, it is clear that there will be found some positive correlationship between water depth and accumulation of such organic constituents as carbon or nitrogen. However the examination on the ratio of carbon to nitrogen showed that it

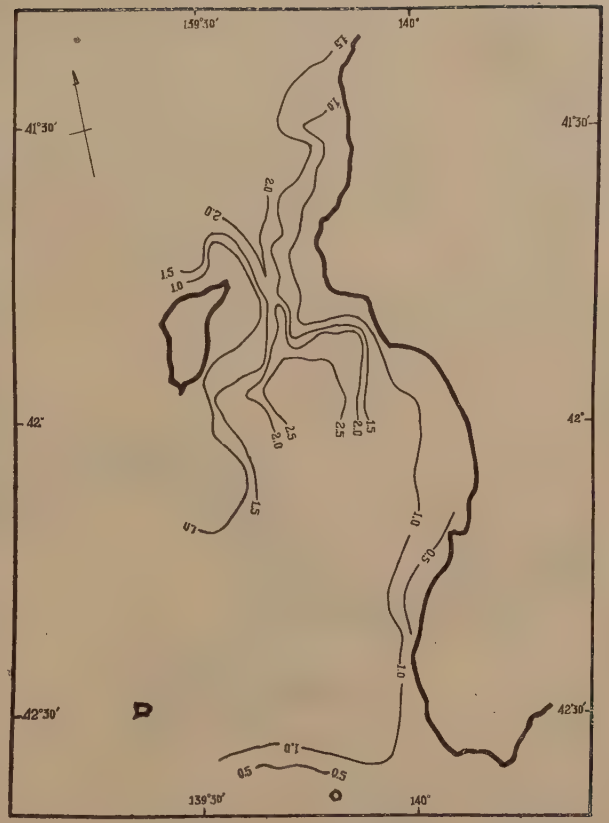


Fig. 4. Distribution of organic carbon content in sediment (%)

Fig. 5. Distribution of total nitrogen content in sediment (%)

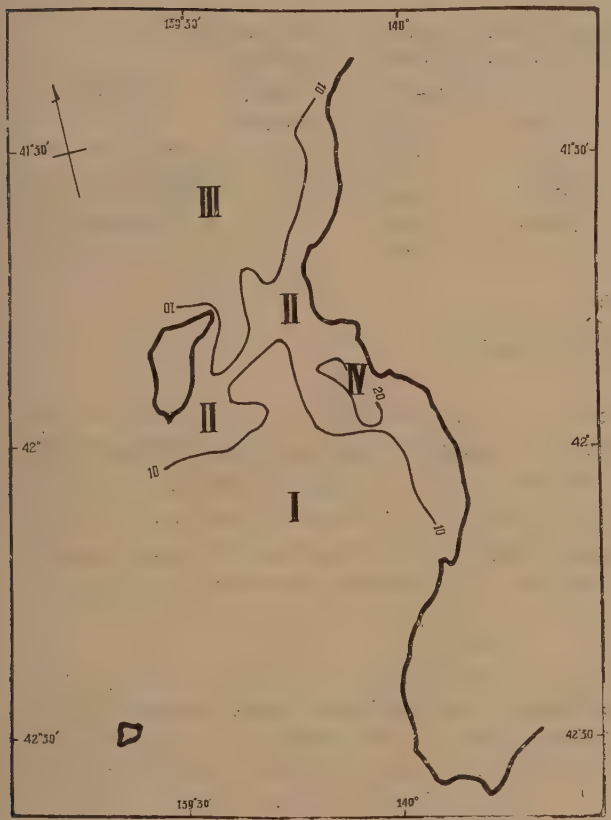


Fig. 6. Distribution of the ratio of carbon to nitrogen in sediment

might be hard to find any correlation to the water depth. Although no correlationship of that ratio to the depth was found, a further examination would show that the regional variation of the ratio as illustrated in Fig. 6 was rather reasonable in relation to the environments of organic accumulation. Ratios higher than 10 were observed on the samples from the shelf in the vicinity of Okushiri Strait (II and IV), but rather low ratios were obtained from the bottom samples of the other open areas (I and III). One may notice in particular that some remarkably large ratios near or over 20 were observed in the samples from the eastern side of the strait (IV).

Rather high ratio suggests the possibility of supplies of fresh terregenous organic substances or progressive decomposition of nitrogenous substances by marine bacteria. Waksman (1938) has shown that fresh organic matter attacked by bacteria will first lose nitrogen than carbon until a more or less definite equilibrium ratio is attained. On the bottom floor at Okushiri Strait, not only was there a fairly good amount of carbonous substances, but nitrogenous substances in sediments would be decomposed more progressively by bacteria than non-nitrogenous ones owing to the sufficient supply of oxygen in the water judging from fairly good sorting of sediments.

At the eastern side of the strait where irregularly large ratios were observed, abundant accumulation of carbonous substances might be somewhat affected by floating fragments of sea weeds which had grown thick in the neighboring coast. The above accumulation would indicate that a small eddy current used to be induced by a branch of Tsushima Current flowing northwards.

Table 2. Organic contents in the sediments and their relations to the water depth of the site of deposition (a=0.05)

	Number of samples	Population mean (x)	Standard deviation (s)	Correlation coefficient in sample (γ)	Correl. coef. in population	Equation of regression*
Org.-C	44	1.143-1.303%	0.260%	0.569	$\rho \neq 0$	C=0.00201d+0.296
Total-N	44	0.124-0.140%	0.016%	0.663	$\rho \neq 0$	N=0.00020d+0.0634
C / N	44	7.89-10.75	4.64	0.014	$\rho = 0$	.....

* C : Carbon content (%)
N : Nitrogen content (%)
d : Water depth of the site of deposition (m)

The author is much indebted to Prof. N. Suzuki of Hokkaido University and Mr. K. Ogaki, Chief of Hokkaido Regional Fisheries Research Laboratory for giving facilities for this work. He is also much obliged to prof. T. Tamura of Hokkaido University for his cooperation in the working out of the biological problems. The present work was rendered possible through the financial support of a grant-in aid for Fundamental Scientific Research from the Ministry of Education, to which the author expresses his sincere thanks.

References

1) Kato, K. & Ishizuka, T. (1949). Chemical studies on marine deposits. Part 1. On the distribution of humus contents in marine deposits in Mutsu Bay. *Jour. Fisheries* (Japan) **54**, 7-12.

2) ——— & Okuda, T. (1951). Chemical studies on marine deposits. Part 3. On the distribution of marine humus contents in the sea to the northwest of Hokkaido Island. *Bull. Fac. Fish., Hokkaido Univ.* **2**, 10-30.

3) ——— (1955). Sedimentological examination on the relation of organic accumulation in sea bottom to the oceanographical environments. *Records Oceano. Works in Japan, N. S.* **4**. (in press).

4) Ogaki, K., Kosugi, H. & Tomabechi, Y. (1953). Submarine topography and distribution of bottom sediment on deep sea fishing ground of Hokkaido. *Bull. Hokkaido Region. Fish. Lab.* **9**, 1-16.

5) Waksman, S. A. (1933). On the distribution of organic matter in the sea bottom and the chemical nature and origin of marine humus. *Soil Sc.* **36**, 125-147.

6) ——— (1938). On the oxidation of organic matter in marine sediments by bacteria. *Jour. Mar. Res.* **1**, 101-118.

Table 1. General properties of bottom samples from the northern Japan Sea in the vicinity of Ōkushiri Strait (August 23–31, 1952)

Station number	Location		Water Depth	Texture of sample				Organic content (%, in dry basis)				Organic remains under microscope	
	Lat.N	Long.E	(m)	Sight	Md	So	Sk	Org-C	Total-N	C/N	Ignition loss	Diatom residue	Silicious sponge
2	42°36.5′	139°48.2′	540	M S	0.075	3.542	0.270	1.73	0.193	8.91	.....	A	
5	42°37.2′	139°47.2′	550	M S	.....	.....	.....	1.27	0.128	9.91	.....	R	
9	42°31.6′	139°46.4′	400	S m	0.096	1.594	0.960	1.16	0.093	12.46	5.16	R R	C
11	42°30.5′	139°45.4′	500	M S	.....	.....	.....	0.97	0.112	8.66	6.15	A	C
13	42°29.4′	139°44.8′	400	M S	.....	.....	.....	1.08	0.113	9.55	.....	R	A
15	42°27.8′	139°44.9′	620	M S	.....	.....	.....	1.54	0.175	8.80	.....	C	C
24	42°24.2′	139°43.0′	550	M	.....	.....	.....	1.66	0.168	7.88	7.22	A	A
28	42°19.2′	139°39.8′	660	M S	.....	.....	.....	1.64	0.213	7.69	8.15	C	C
30	42°16.4′	139°31.6′	700	M S	.....	.....	.....	0.86	0.102	8.43	.....	R	
32	42°16.4′	139°30.0′	550	M S	.....	.....	.....	1.56	0.071	9.62	.....	A	
34	42°16.8′	139°39.6′	480	M S	0.114	1.718	0.845	1.35	0.095	14.20	6.28	C	C
37	42°17.9′	139°39.1′	520	M	.....	.....	.....	1.95	0.168	12.60	8.85	C	C
41	42°13.2′	139°40.6′	540	M S	.....	.....	.....	1.50	0.102	14.70	6.44	R	C
45	42°10.9′	139°36.9′	550	M S	.....	.....	.....	0.86	0.168	5.17	5.29	C	
46	42°11.3′	139°41.2′	580	M	.....	.....	.....	2.07	0.158	13.10	.....	A	C
53	42°09.5′	139°42.4′	380	M	.....	.....	.....	1.13	0.131	8.62	4.72	R	C
54	42°08.2′	139°38.5′	600	M S	0.078	3.685	0.321	1.41	0.126	11.19	5.98	A A	C
56	42°02.2′	139°57.2′	330	S m	.....	.....	.....	1.23	0.052	23.57	4.75	R	A
58	42°02.2′	139°00.2′	160	S m	.....	.....	.....	0.97	0.080	12.12	.....		
61	42°02.5′	139°50.4′	820	M	0.051	6.582	0.150	2.46	0.193	12.16	9.28	R	A
62	42°00.9′	139°38.2′	680	M	.....	.....	.....	1.53	0.161	9.50	7.77	R	
63	42°00.4′	139°34.8′	610	M S	0.096	4.438	0.214	1.53	0.141	10.85	6.34	C	C
67	42°07.9′	139°34.4′	450	M S	.....	.....	.....	1.04	0.118	8.81	.....	C	A
69	42°05.2′	139°33.4′	460	M S	.....	.....	.....	1.31	0.109	11.90	5.11	C	C
70	42°05.8′	139°37.0′	660	M	.....	.....	.....	1.33	0.184	7.23	5.28	C	C
71	42°07.5′	139°47.0′	640	M	0.002	8.944	20.000	2.25	0.116	19.31	7.56	C	C
72	42°07.8′	139°50.8′	330	M S	.....	.....	.....	2.14	0.106	20.17	.....	C	A
75	42°04.8′	139°50.0′	700	M	.....	.....	.....	2.03	0.187	10.52	8.52	C	C
76	42°03.5′	139°39.9′	770	M S	.....	.....	.....	2.87	0.230	12.46	.....	C	A
77	42°02.6′	139°34.9′	620	M S	.....	.....	.....	1.87	0.168	11.18	.....	C	C
86	41°54.1′	139°35.3′	480	M S	.....	.....	.....	0.85	0.112	7.72	.....	C	
88	41°53.4′	139°28.7′	580	M	.....	.....	.....	0.73	0.132	5.60	9.00	C	C
91	41°56.5′	139°30.0′	70	S m	.....	.....	.....	0.87	0.114	7.25	.....	C	A
92	41°57.0′	139°34.5′	400	S M	.....	.....	.....	1.06	0.115	8.41	.....	R	A
94	42°00.3′	139°56.6′	730	M	.....	.....	.....	1.26	0.212	5.94	8.57	R	A
107	41°54.5′	140°00.8′	300	G M S	.....	.....	.....	0.94	0.101	9.30	.....		
118	41°41.6′	139°59.0′	90	S	0.582	1.507	0.876	0.21	0.034	6.17	3.04	R R	
119	41°41.4′	139°57.3′	410	S m	0.219	2.236	0.704	0.61	0.105	5.80	3.53	R	C
121	41°39.3′	139°56.7′	470	S m	0.250	3.030	0.547	1.14	0.126	9.04	5.40		
127	41°33.4′	139°54.9′	600	M	0.083	8.031	0.150	1.39	0.180	7.72	7.96	C	C
135	41°25.8′	139°54.4′	520	M	0.072	9.083	0.092	1.09	0.229	4.75	8.48	(R)	C
148	41°22.5′	139°40.0′	400	G S m	.....	.....	.....	0.46	0.075	6.13	1.39	R	C
159	41°19.8′	139°37.5′	300	G M m	.....	.....	.....	0.32	0.058	5.51	.....	R R	

海藻の窒素同化機構に関する研究—VI

海藻の部位及び体長による硝酸還元酵素作用力の消長

高木光造・村田喜一
(北海道大学水産学部水産食品化学教室)

Studies on the Mechanism of Nitrogen Assimilation in Marine Algae—VI

The variation of nitrate reductase activity according to the portions and to the frond length of some marine algae

Mitsuzo TAKAGI and Kiichi MURATA

Abstract

The variation of the nitrate reductase activity according to the portions and to the frond length of some marine algae was determined. The results obtained are summarized as follows:

1. The nitrate reductase activity of *Undaria pinnatifida* f. *distans* and *Alaria crassifolia* is the strongest in the stipe, followed by holdfast. Next come the transition region and mid-rib. Sporophyll and frond proved to be the weakest in its activity among the various portions.

2. The shorter the frond length, the stronger is *Enteromorpha Linza* in the nitrate reductase activity. Accordingly the activity is gradually reduced with growth.

海藻では褐藻類の一部を除き，陸上植物に見られる如き根，茎，葉の形態学的分化が明瞭でなく，且その生理的機能は環境の相違により自ら異る。即ち根は岩礁に附着して体を支えるに過ぎず，各種栄養塩は専ら藻面より滲透圧の相違により摂取されると一般には認められている。故に生体反応を触媒する酵素の存在は葉に限られ，その作用力も強いことが予想される。

この予想の下に形態学的分化の明瞭なナンブワカメ及びチガイソを用いて各部位における硝酸還元酵素作用力を測定したところ意外に茎，次いで根においてその作用力の強いことを認めたのでその結果について報告する。因みにウスバアオノリを用いて体長による酵素作用力の消長を検したのでその結果についても報告する。

実験方法

(1) 硝酸還元酵素液の調製

前報[1]に記載した方法による。

(2) 硝酸還元酵素作用力測定法

Thunberg tube	Composition of exp. solution	
Side chamber	-0.011M KNO_3 solution	1cc
	-0.52M Sodium lactate solution	1
Main chamber	-M/15 Sørensen's phosphate buffer solution	5
	-Enzyme solution	2
	-Water	1
		Total 10cc

昭和30年4月　日本水産学会年会（東京）に於て講演

上述の内容液を20°C, 10mm以下の真空度で1時間反応させたのち，20% Trichloracetic acid 0.5cc, 活性白土1gを加え，遠心分離してえられる上澄液を分取し，Griess—Ilosvay 試薬2ccを加え，40°C, 15分間加温して生ずる桃色の色調を光電比色計により3.5mmのセル，500mμのフィルターを用いて透過率を測定し，それより生成された亜硝酸イオン量を求める。

実 験 結 果

(1) 部位による硝酸還元酵素作用力の消長

試料は形態学的分化の明瞭なナンブワカメ (*Undaria pinnatifida* f. *distans*) とチガイソ (*Alaria crassifolia*) を用いた。而して各部位はFig. 1に示す如くである。

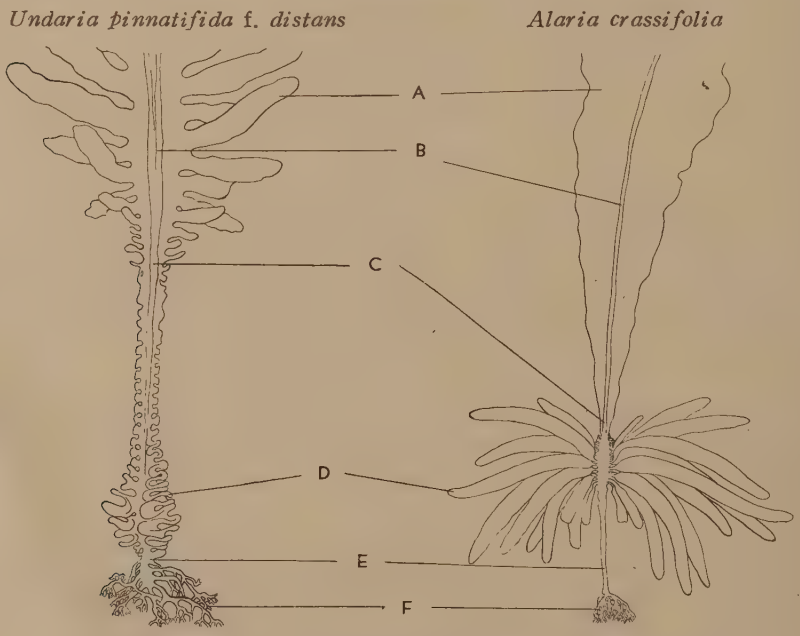


Fig. 1. The portions of *Undaria pinnatifida* f. *distans* and *Alaria crassifolia*, used in the present examinations. After Miyabe.

A: Frond B: Mid-rib C: Transition region
D: Sporophyll E: Stipe F: Holdfast

A. ナンブワカメ (*Undaria pinnatifida* f. *distans*)

5月24日，七重浜にて採取，水温12.0°C

先ずナンブワカメについて各部位における硝酸還元酵素作用力を測定した結果をTable 1に示した。

Table 1. Variation of nitrate reductase activity according to the portions of *Undaria pinnatifida* f. *distans*

No.	Portion	Transmission (%)	NO_2^- produced (μg)	Remarks
1	Frond	93.0	1.36	high viscosity
2	Mid-rib	91.0	1.88	moderate viscosity
3	Transition region	90.5	2.00	high viscosity
4	Sporophyll	92.0	1.68	same as above
5	Stipe	81.2	4.56	moderate viscosity
6	Holdfast	87.5	2.80	clear, non viscosity

Table 1 に示される如くナンブワカメにおいては硝酸還元酵素作用力は茎において最も強いことが認められ，次いで根が強く，移行部，中肋はその次に位し，成実葉及び葉はその作用力が最も弱かつた。もとより葉及び成実葉の酵素液は特に粘稠性であつたので粘質物が酵素反応を幾分阻害したことも事実であろう。

尚供試した成実葉は胞子造成器官であるが未成熟であつた。又生長点である移行部においても特に著しい硝酸還元酵素作用力を認めえなかつた。

B．チガイソ（*Alaria crassifolia*）

5月26日，立待岬にて採取，水温 12.0°C

次にチガイソについて同様の実験を試みてえたる結果をTable 2 に示した。

Table 2. Variation of nitrate reductase activity according to the portions of *Alaria crassifolia*

No.	Portion	Transmission (%)	NO_2^- produced (μg)	Remarks
1	Frond	95.0	0.90	non viscosity
2	Mid-rib	90.6	2.04	same as above
3	Transition region	90.0	2.10	same as above
4	Sporophyll	94.0	1.10	high viscosity
5	Stipe	84.5	3.66	non viscosity
6	Holdfast	88.5	2.55	same as above

Table 2 に示される如くチガイソにおいても前と全く同様の事実を認めた。而してこの場合酵素液は成実葉を除いていずれも非粘稠性であつた。

以上によりナンブワカメ或いはチガイソの茎が海藻の硝酸還元酵素を単離するには好材料と思われる。

(2) 体長による硝酸還元酵素作用力の消長

次にウスバアオノリ（*Enteromorpha Linza*）を用いて体長による硝酸還元酵素作用力の消長を測定した。

C．ウスバアオノリ（*Enteromorpha Linza*）

5月27日，七重浜にて採取，水温 12.0°C

もとより同一時期においても環境条件の相違により，又個体相互間における発生の遅速により生長発育の度合には著しい差異が認められるので，ウスバアオノリの体長を次の如く区分してその作用力を測定し，Table 3 に示した。

Table 3. Variation of nitrate reductase activity according to the frond length of *Enteromorpha Linza*

No.	Frond length (cm)	Transmission (%)	NO_2' produced (μg)
1	2 ± 1	90.5	2.07
2	5 ± 1	92.0	1.68
3	9 ± 2	93.5	1.27
4	14 ± 2	95.5	0.75
5	20 ± 3	96.5	0.50

Table 3 に示される如くウスバアオノリの体長の短かい幼体程硝酸還元酵素作用力の強いことを認めた。

要　　約

海藻の部位及び体長による硝酸還元酵素作用力の消長を測定した結果

1. ナンブワカメ及びチガイソの硝酸還元酵素作用力はいずれも茎が最も強く，次いで根が強く，移行部，中肋はその次に位し，成実葉及び葉は最も弱かつた。

2. ウスバアオノリの硝酸還元酵素作用力は体長の短かい幼体程強く，生長と共に漸減する。

海藻の部位について御教示下された本学部近江彦栄助教授に謹みて謝意を表する。

文　　献

1) 高木光造・村田喜一 (1954). 北大水産彙報 4, 296.

海藻の窒素同化機構に関する研究—Ⅶ

硝酸還元酵素に対する阻害剤の影響

高木光造・村田喜一
（北海道大学水産学部水産食品化学教室）

Studies on the Mechanism of Nitrogen Assimilation in Marine Algae—Ⅶ

Effect of various inhibitors on nitrate reductase

Mitsuzo TAKAGI and Kiichi MURATA

Abstract

The effect of various inhibitors on the nitrate reductase activity of *Ulva pertusa* and *Enteromorpha Linza* has been examined. The results obtained are summarized as follows:

The activity is inhibited markedly by 10^{-3}mol concentration of potassium cyanide and hydroxylamine, but no inhibition is observed at any concentration of potassium fluoride. From the fact that nitrate reductase activity is inhibited by potassium cyanide and hydroxylamine, it may be considered to have a metal constituent.

In addition, nitrate reductase activity is also inhibited by monojodacetic acid, specific inhibitor to the enzymes containing sulfhydryl group. This fact seems to indicate that the inhibition would extend to lactic dehydrogenase, another position in the enzyme system in which nitrate reductase takes part.

Experiments whether the inhibition of nitrate reductase with monojodacetic acid is restored or not by adding cysteine have been tried, but frustrated in this plan on account of the disturbance by excessively addition of cysteine to the point of causing the color reaction resulting from nitrite ion.

海藻は海水中の硝酸塩を窒素源として利用しているに拘らず硝酸還元酵素の存在，性質及びそれらの窒素源を基にして複雑な含窒素有機物を体内で合成する機構については未だ明らかでない。著者[1]等は海藻における硝酸還元酵素の存在を確認し，引続きその性質について究明してきたが，本報においては硝酸還元酵素の本態並びに酵素系を明らかにする目的で各種阻害剤による阻害実験を試みたのでその結果を報告する。

実験方法

(1) 硝酸還元酵素液の調製

前報[1]に記載した方法による。

(2) 硝酸還元酵素の阻害実験法

Thunberg tube	Composition of exp. solution	
Side chamber	0.011M KNO_3 solution	1cc
	0.52M Sodium lactate solution	1
Main chamber	$^{M}/_{15}$ Sørensen's phosphate buffer solution	5
	Enzyme solution	2
	Inhibitor solution	1
		Total 10cc

昭和30年4月　日本水産学会年会（東京）に於て講演

阻害剤としては KCN, NH_2OH, ICH_2COOH 及び KF の $1 \sim 10^{-5}$M 溶液を 1 cc宛反応液中に加えた。かくするときは阻害剤の濃度は反応液に対し $10^{-1} \sim 10^{-6}$M となる。この反応液を 20°C, 10mm 以下の真空度で 1 時間反応させたのち 20% Trichloracetic acid 0.5cc, 活性白土 1 g を加え，はげしく振盪したのち遠心分離してえられる上澄液を分取し，Griess—Ilosvay 試薬 2 cc を加え 40°C, 15分間加温して生ずる桃色の色調を光電比色計により透過率を測定し，それより生成された亜硝酸イオン量を求め，阻害剤を加えないときの亜硝酸イオン量と対比して阻害率(%)を求めた。

実 験 結 果

A. 各種阻害剤による阻害作用

試料はアナアオサ (*Ulva pertusa*) 及びウスバアオノリ (*Enteromorpha Linza*) でえられた結果を Fig. 1 ～2 に示した。

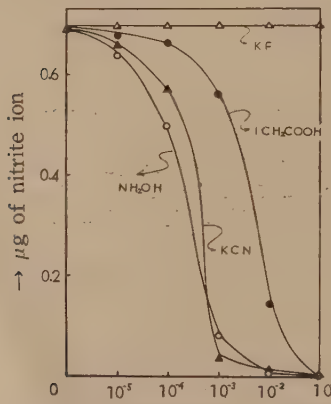


Fig. 1. Inhibition of nitrate reductase in *Ulva pertusa* by various concentrations of inhibitors

▲—▲ KCN ○—○ NH_2OH
●—● ICH_2COOH △—△ KF

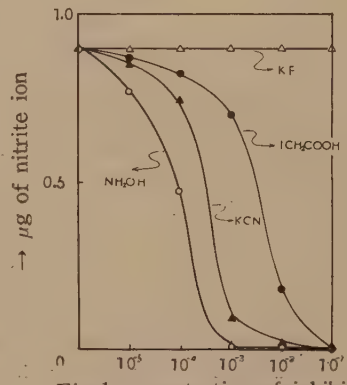


Fig. 2. Inhibition of nitrate reductase in *Enteromorpha Linza* by various concentrations of inhibitors

▲—▲ KCN ○—○ NH_2OH
●—● ICH_2COOH △—△ KF

これを阻害剤を加えないときの亜硝酸イオン量と対比して各阻害剤の 10^{-3}及び 10^{-2}M溶液における阻害率を求めて Table 1 に示した。

Table 1. Per cent of inhibition of nitrate reductase activity in *Ulva pertusa* and *Enteromorpha Linza* by 10^{-3} and 10^{-2} mol concentration of inhibitors

Species	*Ulva pertusa*								*Enteromorpha Linza*							
Inhibitors	KCN		NH_2OH		ICH_2COOH		KF		KCN		NH_2OH		ICH_2COOH		KF	
Final concentration (M/L)	10^{-3}	10^{-2}	10^{-3}	10^{-2}	10^{-3}	10^{-2}	10^{-3}	10^{-2}	10^{-3}	10^{-2}	10^{-3}	10^{-2}	10^{-3}	10^{-2}	10^{-3}	10^{-2}
Inhibition (%)	94	97	88	100	19	83	0	0	90	98	100	100	22	80	0	0

即ち Table 1 に示される如くアナアオサ，ウスバアオノリいずれにおいても硝酸還元酵素作用は KCN, NH_2OH 及び ICH_2COOH によつて阻害されたが，KF によつては全く阻害されなかつた。而して KCN,

NH_2OH は10^{-3}Mで酵素作用を著しく阻害するが，ICH_2COOH は10^{-3}Mではあまり阻害せず，10^{-2}M において酵素作用を著しく阻害する。

KCN，NH_2OH によつて阻害されることから硝酸還元酵素は一種の金属酵素であることが解る。又同時にSH 酵素の特異阻害剤である ICH_2COOH によつても酵素作用が阻害されたが，これは硝酸還元酵素の阻害剤と考えるよりはむしろ硝酸還元酵素の関与する酵素系の他の部分即ち脱水素酵素の作用を阻害したものと思われる。

B．ICH_2COOH による阻害作用の Cysteine 添加による賦活

ICH_2COOH によるSH酵素の阻害作用は Cysteine の添加により賦活されることが知られているので，硝酸還元酵素系にある脱水素酵素作用を阻害すると思われる ICH_2COOH の阻害が Cysteine の添加により賦活されるならば亜硝酸イオンの生成量が増加する筈である。この意図の下にウスバアオノリの硝酸還元酵素液に ICH_2COOH を加えて1時間反応を阻害させたのち，2倍濃度の Cysteine を添加したものとしなかつたものについて亜硝酸イオンの生成量を比較した。

Table 2 はそのときの実験結果を示したもので，Fig. 3 はこれを図示したものである。

Table 2. Inhibition of nitrate reductase in *Enteromorpha Linza* by monojodacetic acid and its restoration by cysteine

No.	Final concentration of ICH_2COOH (M/L)	Concentration of cysteine added (M/L)	Incubating time (hrs)	Transmission (%)	NO_2' produced (μg)
1	0	0	1	95.0	0.90
2	0	0	2	94.0	1.08
3	10^{-3}	0	1	95.7	0.70
4	10^{-3}	0	2	95.3	0.80
5	10^{-3}	2×10^{-2}	2	100	0
6	10^{-2}	0	1	98.3	0.18
7	10^{-2}	0	2	97.7	0.23
8	10^{-2}	2×10^{-1}	2	100	0
9	10^{-1}	0	1	100	0
10	10^{-1}	0	2	100	0
11	10^{-1}	2×1	2	100	0

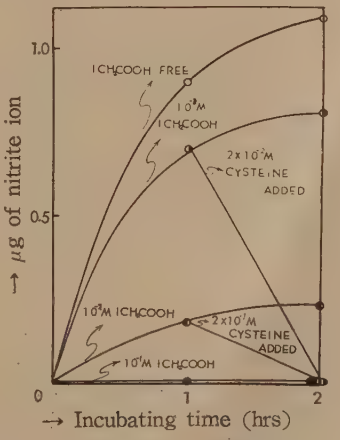


Fig. 3. Inhibition of nitrate reductase in *Enteromorpha Linza* by monojodacetic acid and its restoration by cysteine

Fig. 3 に示される如く Cysteine を添加したものはいずれも亜硝酸イオンの生成量が増加しないのみでなく，却つて0になつた事実から Cysteine そのものが Griess—Ilosvay 試薬による亜硝酸イオンの呈色反応を阻害したものと考えられる。

考　　察

海藻の硝酸還元酵素作用がジャガイモの塊茎のアルデヒド脱水素酵素[2]や，大豆のモヤシの乳酸脱水素酵素[3]の如く，海藻の脱水素酵素が単独で硝酸塩を亜硝酸に還元するのか，或いは江上等[4]の云う如く硝酸還元酵素が存在して蟻酸，コハク酸，アラニンのような水素供与体からとれてきた水素がいくつかの中間水素伝達体を経て最後の水素受容体である硝酸への水素の授受を触媒するのかについての間接的証明として各種阻害剤による阻害実験を行つた。

その結果硝酸還元酵素作用はKCN及びNH_2OHでは$10^{-3}M$濃度で著しく阻害され，ICH_2COOHでは$10^{-3}M$濃度で阻害は僅かであり，$10^{-2}M$に至つて著しく阻害が認められたが，KFでは如何なる濃度においても阻害が全く認められなかつた。渡辺[5]はさきに海藻の脱水素酵素について研究し，このものは5%Ethylurethanによつて阻害が起るが，$10^{-2}M$ KCN, NaF及び$10^{-3}M$ ICH_2COOHによつて起らないことを認めている。

故に著者等の実験結果から$10^{-2}M$ KCNにて阻害されるような本来の意味での硝酸還元酵素の存在することを容易に推定しえられる。而して KCN, NH_2OHによつて阻害されることから海藻の硝酸還元酵素は一種の金属酵素であると推定される。

更に江上等[4]によると大腸菌の硝酸還元酵素は重金属酵素であつて恐らくは鉄酵素であろうと推定し，又A. Nason,[6][7,8] H. J. Evans等[8]は最近植物の硝酸還元酵素について研究し，*Neurospora crassa*及び大豆の葉から硝酸還元酵素を抽出し，いずれもFAD及び恐らくはMoを含む金属酵素であつてTPNの還元型（大豆の葉の酵素ではDPNでもよい）とNO_3イオンの間に作用すると述べている。海藻の硝酸還元酵素が如何なる金属を含む酵素であるかは未知であるが，今後本酵素の単離精製を行つてこの点を究明したいと考えている。

要　　約

アナアオサ及びウスバアオノリの硝酸還元酵素作用力に及ぼす種々阻害剤の影響を実験し，次の結果をえた。即ち酵素作用力は$10^{-3}M$ KCN及びNH_2OHによつて著しく阻害されるが，KFによつては如何なる濃度においても阻害されない。KCN及びNH_2OHによつて阻害されることから一種の金属酵素であろうと示唆した。

又SH酵素の特異阻害剤であるICH_2COOHによつても阻害されるが，これは硝酸還元酵素の関与する酵素系の他の部分即ち乳酸脱水素酵素を阻害するものと推定した。硝酸還元酵素のICH_2COOHによる阻害がCysteineにより賦活されるか否かを試みたが成功しなかつた。

文　　献

1) 高木光造・村田喜一（1954）. 北大水産彙報 **4**, 296；**4**, 306；**4**, 310；**5**, 173；**5**, 176.
2) Michlin, D. (1928). *Biochem. Z.* **202**, 329.
3) ———— & Severrin, B. (1931). *Ibid.* **237**, 339.
4) 江上不二夫・佐藤了（1947）. 日化誌 **68**, 39；(1948). **69**, 160；(1949). **70**, 297；(1949). *Bull. Chem. Soc. Japan* **22**, 137.
5) Watanabe, A. (1949). *Acta Phytochim.* **15**, 129.
6) Nason, A. & Evans, H. J. (1953). *J. Biol. Chem.* **202**, 655.
7) Nicholas, D. J. D., Nason, A. & McElroy, W. D. (1954). *Ibid.* **207**, 341.
8) ———— & ———— (1954). *Ibid.* **207**, 353.

海藻の窒素同化機構に関する研究—Ⅷ

硝酸還元酵素の中間水素伝達体に就て

高木光造・村田喜一
(北海道大学水産学部水産食品化学教室)

Studies on the Mechanism of Nitrogen Assimilation in Marine Algae—Ⅷ

On the electron carrier of nitrate reductase in *Porphyra yezoensis*

Mitsuzo TAKAGI and Kiichi MURATA

Abstract

Experiments were made to ascertain whether methylen blue, coenzyme 1(DPN) and cytochrome C are able or unable to fulfil a specific function as an electron carrier of nitrate reductase in *Porphyra yezoensis*. The results obtained are summarized as follows:

1. Methylen blue can stand as a representative of an electron carrier of nitrate reductase.
2. Cytochrome C is one of the real electron carriers of nitrate reductase.
3. Coenzyme 1(DPN) is not separately able to act as an electron carrier of nitrate reductase.

硝酸還元酵素の中間水素伝達体としては，水素供与体や菌の種類によつていろいろのものが使われるが，CoI (DPN) や yellow enzyme (Flavoproteid), 更に少くとも大腸菌においては Cytochrome b_1 などが関与している事実が明らかにされた。

又丹羽・佐藤等[1]は大腸菌による硝酸の還元には少くとも2種の酵素の協力が必要で，その一つは還元基質に対する脱水素酵素であり，他の一つは硝酸還元酵素であつてこの二つの酵素の間に山県の説く如く Mb (methylen blue) で代用しうるような何らかの中間水素伝達体の存在することを述べている。

著者等の実験においてもほゞ同様の機作によつて硝酸の還元が行われることが予想されたので，Mbが大腸菌の場合と同様に硝酸還元酵素の中間水素伝達体の役目を代用しうるか，又アサクサノリ(*Porphyra tenera*)中に Cytochrome C の存在が薬師寺[2]により指摘確認されているので，Cytochrome C が海苔における硝酸還元酵素の真の中間水素伝達体となりうるか，更に又乳酸脱水素酵素の助酵素である CoI の添加により海苔の硝酸還元酵素作用力の増進が見られるか，これらの点について検討した結果を報告する。

実験方法

(1) 硝酸還元酵素液の調製

前報[3]に記載した方法による。

(2) 硝酸還元酵素作用力測定法

前報[3]に記載した方法による。

実験結果

A. 硝酸還元酵素に対する Mb の影響

昭和30年4月　日本水産学会年会（東京）に於て講演

供試海藻は6月3日，七重浜にて採取したスサビノリ（*Porphyra yezoensis*）を用いた。又Mbの濃度は 2×10^{-4}M 溶液を用い，その量を変えて反応液に加えたときの実験結果を Table 1 に示した。

Table 1. Effect of graded amounts of methylen blue on nitrate reductase activity of *Porphyra yezoensis*

Side chamber	0.011M KNO_3 soln.	1	1	1	1	1	1	1	1	1	1
	0.52M Sodium lactate soln.	1	1	1	1	1	1	1	1	1	1
Main chamber	pH 7.17 $^{M}/_{15}$ Sørensen's phosphate buffer soln.	5	5	5	5	5	5	5	5	5	5
	Enzyme soln.	2	2	2	2	2	2	2	2	2	2
	Methylen blue (2×10^{-4}M)	0	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9
	Water	1	0.9	0.8	0.7	0.6	0.5	0.4	0.3	0.2	0.1
Transmission (%)		90.1	89.8	89.5	89.0	88.3	88.2	88.5	89.5	90.0	90.1
NO_2' produced ($\mu g/_{1h}$)		2.08	2.18	2.25	2.40	2.60	2.63	2.55	2.25	2.10	2.08

Table 1 によつて示される如く Mbはスサビノリ硝酸還元酵素の中間水素伝達体の役目を代用しうることが解る。しかも反応液を10ccとするとき Mb の最適濃度は 2×10^{-4}M 溶液 0.5ccを添加したときで，このとき生成された亜硝酸イオンの量は最大となつた。

次に硝酸還元酵素の水素供与体として，乳酸ソーダの代りにコハク酸，リンゴ酸，クエン酸，グリシン，L-グルタミン酸を用い，2×10^{-4}M の Mb 0.5ccを添加したときとしなかつたときの亜硝酸イオンの生成量を検してみた。その結果を Table 2 に示した。

Table 2. Effect of methylen blue on nitrate reductase activity of *Porphyra yezoensis* when use is made of succinic-, malic-, citric acid, glycine and L-glutamic acid instead of sodium lactate as hydrogen donator

		1	1	1	1	1	1	1	1	1	1
Side chamber	0.011M KNO_3 soln.	Succinic acid		Malic acid		Citric acid		Glycine		L-Glutamic acid	
	0.2M Hydrogen donators	1	1	1	1	1	1	1	1	1	1
Main chamber	pH7.17 $^{M}/_{15}$ Sørensen's phosphate buffer soln.	5	5	5	5	5	5	5	5	5	5
	Enzyme Soln.	2	2	2	2	2	2	2	2	2	2
	Methylen blue (2×10^{-4}M)	0	0.5	0	0.5	0	0.5	0	0.5	0	0.5
	Water	1	0.5	1	0.5	1	0.5	1	0.5	1	0.5
Transmission (%)		96.0	94.8	96.5	96.5	99.0	99.0	99.0	99.0	98.0	98.0
NO_2' produced ($\mu g/_{1h}$)		0.60	0.92	0.45	0.45	0.05	0.05	0.05	0.05	0.17	0.17

Table 2 によつて示される如く，コハク酸を水素供与体として用いたときにはMbは乳酸ソーダと同様に硝酸還元酵素の中間水素伝達体となりえたが，リンゴ酸，クエン酸，グリシン及びL-グルタミン酸に対しては明確には認められなかつた。

B．硝酸還元酵素の真の中間水素伝達体について

薬師寺[2]は生海苔（*Porphyra tenera*）5 Kgから Cytochrome C 1 gを結晶状に分離した。今仮りにその収量を50%とすると著者等が実験に用いる酵素液 2 cc中には Cytochrome C 約 0.4mg を含むことになる。従つ

て今 Cytochrome C の10^{-5}M 溶液を作製して反応液中に0.2ccを加えると上記 Cytochrome C 量の約6倍量を反応液中に新たに添加したことになる。この考えの下に Cytochrome C の 10^{-3}M 溶液 0.2cc を反応液に加えた。この際同時に等Mの Co I 及び前記 2×10^{-4}M の Mbをもいろいろの組合せで加えてこれらが酵素系に関与するか否かを検討した。そのときの実験結果を Table 3 に示した。

Table 3. Effect of methylen blue, Co I (DPN) and cytochrome C on nitrate reductase activity of *Porphyra yezoensis*

Side chamber	0.011M KNO_3 soln.	1	1	1	1	1	1	1	1
	0.52M Sodium lactate soln.	1	1	1	1	1	1	1	1
Main chamber	pH7.17 M/15 Sørensen's phosphate buffer soln.	5	5	5	5	5	5	5	5
	Enzyme soln.	2	2	2	2	2	2	2	2
	Methylen blue (2×10^{-4}M)	—	—	0.5	—	0.5	0.5	—	0.5
	Coenzyme I (1×10^{-3}M)	—	0.2	—	—	0.2	0.2	0.2	—
	Cytochrome C (1×10^{-3}M)	—	—	—	0.2	—	0.2	0.2	0.2
	Water	1	0.8	0.5	0.8	0.3	0.1	0.6	0.3
Transmission (%)		90.0	90.0	88.5	88.0	88.5	88.5	88.0	88.5
NO_2' produced ($\mu g/1h$)		2.10	2.10	2.55	2.70	2.55	2.55	2.70	2.55

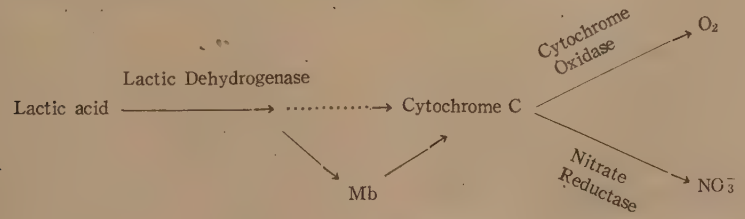
Table 3 に示される如く Co I によつてスサビノリの硝酸還元酵素作用力は増加しないことが解る。而してこれは Co I が単独では海藻の乳酸脱水素酵素の助酵素即ち硝酸還元酵素の中間水素伝達体になりえない事実を意味すると思うが，Co I がこの場合の反応液中に充分含まれていたことによつて，これを新たに添加してもその効力を示さなかつた事も考えられるからこの点は尚吟味の余地があると思われる。

然しながら Mb は前の実験同様に硝酸還元酵素の中間水素伝達体の役目を代用しうることは明らかであり，又 Cytochrome C がスサビノリ硝酸還元酵素の真の中間水素伝達体となりえたので，恐らくこのものがアマノリの窒素同化に実際に関与しているものと思われる。

考　　察

乳酸から脱水素されるときの水素受容体としては従来 Co I (DPN)をとるもの(筋肉の場合)と Cytochrome をとるもの（酵母の場合）との二つの型に限られている。従つて乳酸から脱水素される海藻の硝酸還元酵素系には *Neurospora crassa* の如く Co II (TPN) の関与は考えられない。

著者等は本実験において海藻の硝酸還元酵素は Co I によつては確定的でないが，Mb, Cytochrome C によつてその作用力を増加する事実を認めたことから海藻の乳酸脱水素酵素は酵母と同じ型をとるものではなかろうかと思われる。而して Green, Stickland & Tarr[4] は乳酸から脱水素される硝酸還元酵素系に Cytochrome C が関与しないと述べているが，著者等の実験事実より海藻の硝酸還元酵素系に Cytochrome C が関与していることは疑いない。即ち



最近 A. Nason, H. J. Evans[5] は *Neurospora crassa* から硝酸還元酵素を抽出し，補欠分子族として FAD (Flavin adenine dinucleotide) を含む Mo 酵素であつて Co II の還元型と NO_3 イオンとの間に作用すると述べている。即ち

$$NO_3^- + TPNH + H^+ \xrightarrow{\text{Nitrate Reductase}} NO_2^- + TPN^+ + H_2O$$

更に同氏等[6] は FAD (FMNをも含む) 及び Mo が TPN の還元型から NO_3 イオンに水素が渡される径路に存在して硝酸還元酵素の中間水素伝達体の役目を果すことを提案した。即ち

$$TPNH \longrightarrow FAD\ (or\ FMN) \longrightarrow Mo \longrightarrow NO_3^-$$

一方辻村等[7] は広く海藻中に Flavin の存在を認め，各種海藻中の Flavin 含量を定量して特にアマノリ類に多く含まれることを証しているし，荻野[8] や著者の一人〔高木[9]〕は海藻中に有機塩基として Adenine の広く存在することを認めているので各種海藻中に FAD が存在し，硝酸還元酵素系に実際に関与しているであろうことは充分可能性のあるところである。

従つて海藻の硝酸還元酵素系に果して FAD や Mo が関与するか否かについては今後実験的に究明したいと考えている。

要　　約

Mb, Co I 及び Cytochrome C がスサビノリの硝酸還元酵素の中間水素伝達体になりうるか否かを実験し，次の事を明らかにした。

1. Mb は硝酸還元酵素の中間水素伝達体の役目を代用しうる。
2. Cytochrome C が硝酸還元酵素の真の中間水素伝達体である。
3. Co I は単独では硝酸還元酵素の中間水素伝達体となりえない。

本研究の遂行に当り種々貴重な助言を賜つた本学部斎藤恒行教授に深甚なる謝意を表する。

文　　献

1) 丹羽　允・佐藤　了・谷口茂彦 (1951). 酵素化学シンポジゥム **6**, 40.
2) Yakushiji, E. (1938). *Phytochim.* **8**, 5328.
3) 高木光造・村田喜一 (1954). 北大水産彙報 **4**, 296.
4) Green, D. E., Stickland, L. H. & Tarr, H. L. A. (1934). *Biochem. J.* **28**, 1812.
5) Nason, A. & Evans, H. J. (1953). *J. Biol. Chem.* **202**, 655.
6) Nickolas, D. J. D. & Nason, A. (1954). *Ibid.* **211**, 183.
7) 辻村みちよ・田部井菊子・和田つる (1952). 農化 **26**, 11.
8) 荻野珍吉 (1941). 日水誌 **10**, 156; (1943). **12**, 48.
9) 高木光造 (1952). 北大水産彙報 **2**, 297.

STUDIES ON THE NUTRITIVE VALUE OF THE MEAT OF SEA CUCUMBER (*STICHOPUS JAPONICUS* SELENKA)

V. Nitrogen Distribution and Amino Acid Composition of Protein of Meat of *Stichopus japonicus*

Eiichi TANIKAWA and Hirotoshi ISHIKO
Faculty of Fisheries, Hokkaido University

In order to ascertain the nutritive value of foods, the nitrogen distribution and amino acid composition of the protein in the foods should be determined. This is true because there are nutritive-complete proteins which have special important amino acids and nutritive incomplete which are lacking in the important amino acids. It is fully accepted that the proteins of cattle meat, chicken meat and fish meat are nutritive-complete.

The authors have tried to investigate the nitrogen composition of the so called meat (edible part) of *Stichopus japonicus* in order to determine whether it contains nutritive-complete protein or not.

1. Nitrogen distribution and amino acid composition of protein of raw fresh meat

(1) Sample

Raw fresh *Stichopus japonicus* which was caught in the sea near Hakodate was employed. The bodies of *Stichopus japonicus* were eviscerated and were separated into two parts, meat part and skin part, as described previously.[7] Each part of the bodies was cut with a knife and crushed in a glass mortar respectively and employed as samples.

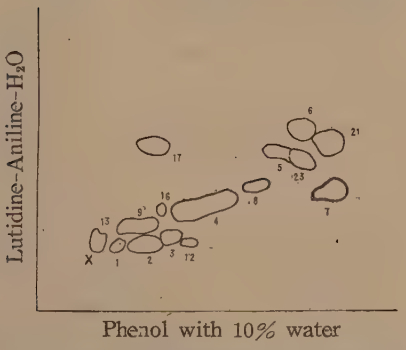


Fig. 1 Paper chromatograms of amino acids detected in spring *Stichopus japonicus* meat (hydrolysis with 20 %HCl)

1. Aspartic acid
2. Glutamic acid
3. Glycine
4. Alanine
5. Valine
6. Phenylalanine
7. Proline
8. Hydroxyproline
9. Serine
12. Ornithine
13. Cystine
16. Threonine
17. 3,5-dijodotyrosine
21. Leucine
23. Methionine

Three to five gram quantities of the prepared samples of each part were hydrolyzed with 120 cc of 20% HCl solution for 48 hours, and then the nitrogen distribution of protein of samples was investigated by the use of Van Slyke's method.

At the same time small quantities of each of the hydrolyzates were employed to determine amino acid composition by two-dimensional paper chromatography. Each hydrolyzate was concentrated on a boiling water bath, and then was spotted on a

corner of a square filter paper. First run (ascending) was done with phenol containing 10% water; after drying a second run was made with the mixed solution of lutidine, aniline and water (65 : 7.2 : 28) in the right angled direction. After drying of the dampened paper, adsorbed amino acids were tested by 1% ninhydrin butanol solution, and the kinds of amino acid were determined by the values of Rf of the colored maps.

(2) Experimental results and discussion

Results obtained are shown as Table 1, Fig. 2 and Fig. 3.

Table 1. Nitrogen distribution of meat and surface skin of *Stichopus japonicus*

Fraction \ Sample	Meat part		Surface skin part	
	g in 100 g of sample	Per cent to total-N	g in 100 g of sample	Per cent to total-N
Total-N	1.020	100.00	0.997	100.00
20% HCl insoluble-N	0.049	4.80	0.053	5.35
20% HCl soluble-N	0.971	95.20	0.944	94.69
Amide-N	0.035	3.43	0.025	2.53
Humine-N	0.115	11.47	0.175	17.55
Basic total-N	0.228	22.40	0.216	21.65
Arginine-N	0.114	11.16	0.104	10.48
Histidine-N	0.095	9.43	0.086	8.61
Lysine-N	0.008	0.82	0.019	1.87
Cystine-N	0.009	0.93	0.007	0.69
Basic amino-N	0.078	7.65	0.080	8.04
Mono amino acid total-N	0.579	56.70	0.534	53.60
Mono amino acid amino-N	0.430	42.15	0.449	45.00
Mono amino acid non amino-N	0.149	14.55	0.085	8.60
Total *	1.008	98.80	1.003	100.60

* (20% HCl insoluble-N)+(Amide-N)+(Humine-N)+(Basic total-N)+(Mono amino acid total-N)

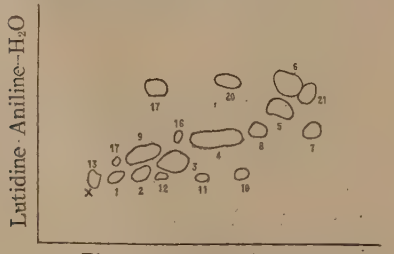


Fig. 2 Paper chromatograms of amino acids detected in surface skin part of spring *Stichopus japonicus* (hydrolysis with 20% HCl)

1. Aspartic acid
2. Glutamic acid
3. Glycine
4. Alanine
5. Valine
6. Phenylalanine
7. Proline
8. Hydroxyproline
9. Serine
10. Arginine
11. Lysine
12. Ornithine
13. Cystine
16. Threonine
17. 3.5-dijodotyrosine
20. Tyrosine
21. Leusine

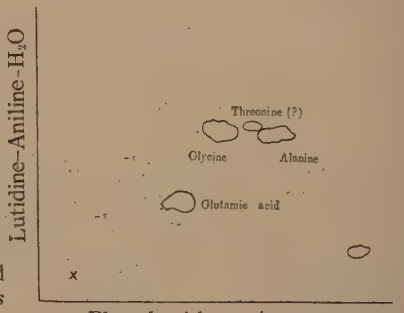


Fig. 3 Paper chromatograms of free amino acids detected in fresh *Stichopus japonicus* meat

As seen in Table 1, the amount of total nitrogen in 100 g of the meat part of *Stichopus japonicus* is larger than that of the skin part. On the other hand, the amount of HCl-insoluble nitrogen of the meat part is smaller than that of skin part. The amount of amide nitrogen of the meat part is larger than that of the skin part having a difference of about 1%. The amount of humine nitrogen of the meat is smaller than that of the skin part with a difference of 6%. The amount of basic nitrogen of the meat part is as large as that of the skin part; the amounts of the both parts indicate 21~22%. The amount of arginine nitrogen of the meat and skin parts indicate respectively 10~11% being the largest quantity among the basic nitrogens. The amount of histidine nitrogen which indicates 8~9% is next in quantity to the amount of arginine nitrogen. The amount of mono-amino nitrogen is 57% for the meat part and 54% for the skin part.

To compare the nitrogen distribution of the meat of *Stichopus japonicus* with other fish meat, the differences are shown as Table 2.

Table 2. Comparative nitrogen distribution in various fish and shell fish meat (calc. as 100 of total-N)

Sample	Squid (dried)	Octopus	Sea-bream	Sardine	Atka mackerel	*Stichopus japonicus*
Researcher	Igarashi & Shirai[1]	Sekine, Sumi & Hara[2]	Hatagoshi[3]	Fujikawa & Naganuma[4]	Fujii[5]	Tanikawa & Ishiko
Year	1953	1920	1932	1936	1953	1953
Total-N (dry matter)	12.60	15.59	16.57	13.31	15.98	9.53
Amide-N	4.09	4.33	8.14*	6.44	5.36	3.43
Humine-N	3.33	1.45	1.81	1.22	5.14	11.47
Basic total-N	39.70	32.00	28.16		26.13	22.40
Arginine-N	20.00	17.10	11.34	11.86	8.65	11.16
Histidine-N	8.74	4.90	0.80	0.41	8.71	9.43
Lysine-N	10.07	9.37	16.19	14.97	7.85	0.82
Cystine-N	0.92	0.57	0.30	0.20	0.91	0.93
Mono amino acid total-N	52.80	59.94	59.94		56.56	56.70
Mono amino acid amino-N	43.00	58.50	59.22	61.38	46.22	42.15
Total	99.92	97.28	98.53		98.85	98.80
Remarks	Based on 100 of hydrochloric acid soluble-N, containing extract	Containing extract	Values below the mark * were calculated to the base on 100 of hydrochloric acid soluble-N 16.47%). Lacking extract.	Lacking extract	Containing extract	Containing extract

The amount of total nitrogen in the meat of *Stichopus japonicus* is about 1%, which is much smaller than the 3% total nitrogen of other fish meat. That is to say, the amount of protein in the meat of *Stichopus japonicus* may be smaller than in other fish meat. However, the ratio of the amounts of various fractions of nitrogen to the amount of the total nitrogen in the meat of *Stichopus japonicus* is almost the same as in other fish meat, excepting a few fractions.

The amounts of basic nitrogen and mono-amino nitrogen of the *Stichopus japonicus* meat are small, but the amount of arginine nitrogen is comparatively large.

In addition, the meat of *Stichopus japonicus* contain lysine and other amino acids which are nutritive-important amino acids. They were also detected by the paper chromatography as later stated.

That is to say, the protein of the meat of *Stichopus japonicus* is lacking in some amino acids which are contained in the fish meat protein.

The meat of *Stichopus japonicus* consists mainly of connective tissue which is collagen and a small amount of other proteins as previously stated. Collagen does not contain cystine, cysteine and tryptophan.

In the present experiment, the meat part was found not to contain tryptophan by paper chromatography, as it was the HCl hydrolyzate.

It is remarkable that the meat of *Stichopus japonicus* contains a large amount of humine nitrogen differing from other fish meat. The significance of this fact can not be interpreted on the basis of data obtained by the present experiments.

The kinds of amino acids in the meat and skin parts which have been detected by paper chromatography are seen as in Fig. 2 and Fig. 3. Those amino acids are aspartic acid, glutamic acid, glycine, alanine, valine, phenylalanine, proline, hydroxyproline, serine, arginine, lysine, ornithine, cystine, threonine, 3-5 dijodotyrosine, tyrosine, leucine and methionine. To state it again, almost all the amino acids are contained in the meat and skin parts of *Stichopus japonicus*.

2. The kind of amino acids in free state in the meat of *Stichopus japonicus*

The free amino acids in the meat of *Stichopus japonicus* were detected by paper chromatography.

(1) Sample

To 10 g of crushed raw meat of *Stichopus japonicus* was added the same volume of dist. water and then mixed homogeneously and left for 30 minutes at room temperature. Protein in the mixture was removed by 5 cc of 20% trichloracetic acid solution.

Free amino acid solution obtained thus was concentrated to 0.5 cc on a water bath of 45°-50°C.

(2) Running and coloring in the paper chromatography

The kind of amino acids was detected by the same two-dimensional paper

chromatography as described above.

(3) Results and discussion

Results obtained are shown in Fig. 4.

As seen in Fig. 4, the kinds of amino acids in free state which were detected in the meat of *Stichopus japonicus* are glycine, alanine, glutamic acid, arginine, and threonine-like substance.

Arginine which was found in large quantity in the hydrolyzate by Van Slyke's method is present in free state in living bodies of *Stichopus japonicus*.

Threonine has been detected in the hydrolyzate of the meat by Hatagoshi[6].

Threonine-like substance was also detected by paper chromatography in free state. But the map of the threonine-like substance appeared near the maps of glycine and alanine and the map of alanine was spread, therefore the map of threonine was not accurately determined.

Histidine, proline, valine, leucine and lysine which have been detected by paper chromatography in free state by other authors were not ascertained in this experiment.

Summary

The ratio of the nitrogen distribution of meat and skin parts of *Stichopus japonicus* was almost equal to that of fish meat except that the amount of humine nitrogen of the former is larger then that of the latter. The amounts of various nitrogen compounds of the former are inferior quantitatively to those of the latter.

The kinds of amino acids of hydrolyzed protein of *Stichopus japonicus* which were detected by partition paper chromatopraphy were as follows: aspartic acid, glutamic acid, glycine, alanine, valine, phenylalanine, proline, hydroxyproline, serine, arginine, lysine, ornithine, cystine, threonine, 3-5 dijodotyrosine, tyrosine, leucine, methionine. However tryptophan is lacking in the *Stichopus japonicus* meat and skin parts.

The kinds of amino acid in free state were detected as follows: glycine, alanine, glutamic acid, arginine and threonine-like substance.

Literature cited

1) Igarashi, H. & Shirai, B. (1953). *Hokkaido Suisan Shikenjo Geppo* **10** (3), 36-44. (in Japanese).
2) Sekine, H., Sumi, H. & Hara, H. (1920). *Suisan Koshusho Shiken Hokoku* **16** (1), 1. (in Japanese).
3) Hatagoshi, Y. (1933). *J. Chem. Soc. Japan* **54**, 982.
4) Fujikawa, R. & Naganuma, H. (1936). *Bull. Jap. Soc. Sci. Fish.* **5** (2), 92-97.
5) Fujii, Y. (1954). *Bull. Fac. Fish., Hokkaido Univ.* **5** (3), 253.
6) Hatagoshi, Y. (1941). *J. Agr. Chem. Soc. Japan* **17** (197), 64-66.
7) Tanikawa, E., Akiba, M. & Yoshitani, S. (1955). *Bull. Fac. Fish., Hokkaido Univ.* **5** (4), 341.

STUDIES ON THE NUTRITIVE VALUE OF THE MEAT OF SEA CUCUMBER (*STICHOPUS JAPONICUS* SELENKA)

VI. Nitrogen Distribution and Kind of Chemical Components of the Extractives of the Meat and Skin Parts of *Stichopus japonicus*

Eiichi TANIKAWA, Hirotoshi ISHIKO and Terushige MOTOHIRO
Faculty of Fisheries, Hokkaido University

1. Nitrogen distribution in the hydrolyzate of the extractive

It is necessary to know the nitrogen distribution of the extractive of meat and skin parts of *Stichopus japonicus* for the sake of the determination of the tasteful matter.

The authors have undertaken to ascertain the nitrogen distribution of nonprotein in the hot water extractive of the meat part and skin part of *Stichopus japonicus*.

In this experiment, each chemical component in the extractive has not been separated.

(1) Preparation of sample

The bodies of *Stichopus japonicus* were divided into meat part and skin part as shown in Fig. 1, previous paper.[4] One hundred and fifty grams of meat and skin parts respectively were extracted with about 500 cc of hot water at 45°–50°C for one hour. Hot water soluble protein in each extractive was precipitated by 20% trichloracetic acid solution, and was filtered. Each filtrate was employed.

(2) Experimental method

Each filtrate was concentrated to about 100cc. Peptide-like substance* which is formed during the concentration of the filtrate was again filtered, and then the filtrate was hydrolyzed with 20% HCl solution for 48 hours. Nitrogen distribution in the hydrolyzate was determined by Van Slyke's method. The amounts of total nitrogen in meat and skin parts were estimated by the usual method.

(3) Experimental results

Results obtained are shown in Table 1.

As seen in Table 1, soluble nitrogen in the meat extractive which was hydrolyzed by 20% HCl solution for about 48 hours is about 0.06 g per 100 g of meat, and there is no difference found between meat part and skin part.

The ratio of amide nitrogen to total nitrogen is 3–5%, and the ratio of humine nitrogen to total nitrogen is 13–17%. The percentage contained in the skin part is larger than that in meat part. The ratio of the amount of histidine nitrogen to the amount of total nitrogen is the largest in the fraction of basic nitrogen showing 20–24%; this component is important to the taste of *Stichopus japonicus*.

* This substance was detected by paper chromatography after the filtration of it. The Biuret reaction showed light violet color. Consequeutly, the substance was determined to be not an amino acid.

Table 1. Nitrogen distribution of extracts from meat and surface skin of *Stichopus japonicus*

Sample / Fraction	Extract of meat part		Extract of surface skin part	
	g in 100 g of sample	Per cent to total-N of extract	g in 100 g of sample	Per cent to total-N of extract
Total-N of extract	0.067	100	0.058	100
20 % HCl insoluble-N	0.003	4.48	0.002	3.45
20 % HCl soluble-N	0.064	95.52	0.056	96.55
Amide-N	0.002	2.98	0.003	5.17
Humine-N	0.009	13.41	0.010	17.21
Basic total-N	0.025	37.35	0.023	39.70
Arginine-N	0.011	16.40	0.008	13.80
Histidine-N	0.014	20.09	0.014	24.15
Lysine-N	0.0004	0.598	0.001	1.72
Cystine-N	0.0003	0.448	0.0003	0.517
Basic amino-N	0.008	11.94	0.008	13.80
Mono acid total-N	0.027	40.3	0.022	37.97
Mono amino acid amino-N	0.010	14.9	0.010	17.24
Mono amino acid non-amino-N	0.017	25.4	0.012	20.73
Total	0.066	98.52	0.060	103.5
Remarks	Total-N of meat part is 1.02 %		Total-N of surface skin part is 0.997 %	

The absolute amounts of total nitrogen of the meat and the meat extractive are smaller than those found in other fish meat. That is to say, the amount of total nitrogen of extractive of meat and skin parts is about 60 mg%, respectively, and this quantity is only a fraction of the quantity of total nitrogen of extract of red flesh fish meat, bonito and tuna[1], or squid meat.[2] The quantity of total nitrogen of extract of *Stichopus japonicus* meat is a quarter of that of white flesh fish meat of tetradon fish, ayu-fish, flat fish, etc. Similar facts are seen not only in the absolute amount of total nitrogen of the extract of the meat of *Stichopus japonicus*, but also in the ratio of the amount of total nitrogen of extractive of the meat part or the skin part to the amount of total nitrogen in meat part or skin part.

The ratio of the amount of total nitrogen of extract of the meat of *Stichopus japonicus* to the amount of total nitrogen thereof is about 6%. On the other hand, the similar percentages of tetradon fish and flat fish are about 10%. The light taste of

Stichopus japonicus may be owing to those facts. Simidu[9] has said that the activity and the heavy taste of red flesh fish may be owing to the large amount of basic nitrogen (diamino acid nitrogen). In fact, red flesh fish meat does have a larger amount of diamino nitrogen than monoamino nitrogen in the extractive. The amounts of diamino nitrogen and monoamino nitrogen in the extractive of the meat of *Stichopus japonicus* are almost equal.

The absolute amounts of monoamino nitrogen and diamino nitrogen in the extractive of the *Stichopus japonicus* meat are smaller than those in other fish. The light taste of the meat of *Stichopus japonicus* may be also due to this fact.

The amount of humine nitrogen in the extract of the *Stichopus japonicus* meat is remarkably large in the meat part as well as in the skin part.

2. The kind of chemical components detected in the extracts of meat and skin parts by paper chromatography

In the experiment described just above, the amounts of chemical components in the extract of meat and skin parts of *Stichopus japonicus* are found to be inferior to those of other fish meat as estimated by Van Slyke's method.

The authors have tried to investigate the kind of chemical components. The extract of meat and skin parts were respectively concentrated and subjected to two-dimensional paper chromatography.

(1) Sample

The sample was prepared by the same method as described in the previous article 1.

(2) Experimental method

Small quantities of the concentrated extract of meat and skin parts respectively were employed for two-dimensional paper chromatography. Adsorbed chemical components were tested by ninhydrin, Pauli, Sakaguchi and Xanthoprotein reactions and by Dragendorf's reagent.

(3) Results

Fig. 1 shows a two-dimensional paper chromatogram which exhibits the kind of chemical components in the extract of meat part of *Stichopus japonicus*. The first run was made with the phenol containing 10% water and second run with a mixture of lutidine, aniline and water (65 : 7.2 : 28). The spots were tested by 1% ninhydrin butanol solution.

As seen in Fig. 1, amino acids which have been found in fish meat are also nearly all found in the extract of meat part of *Stichopus japonicus*.

Fig. 2 shows the paper chromatogram of the extract of the skin part which was obtained by the same method. No difference in the kinds of amino acids in the meat parts and skin parts was detected.

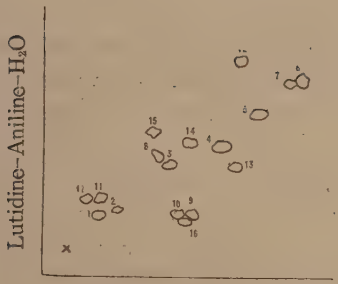


Fig. 1. Paper chromatograms of amino acids of extract from *Stichopus japonicus* meat

1. Aspartic acid
2. Glutamic acid
3. Glycine
4. Alanine
5. Valine
6. Phenylalanine
7. Leucine
8. Serine
9. Arginine
10. Lysine
11. Cystine
12. Tyrosine
13. Arcaine
14. Threonine
15. Betaine
16. Ornithine
17. Adenine

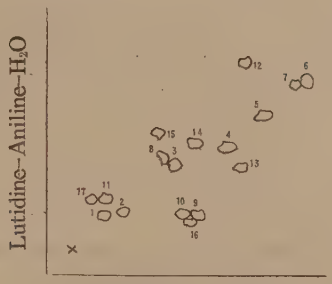


Fig. 2. Paper chromatograms of amino acids of extract from surface skin of *Stichopus japonicus*

1. Aspartic acid
2. Glutamic acid
3. Glycine
4. Alanine
5. Valine
6. Phenylalanine
7. Leucine
8. Serine
9. Arginine
10. Lysine
11. Cystine
12. Tyrosine
13. Arcaine
14. Threonine
15. Betaine
16. Ornithine
17. Adenine

From the extract of meat part and skin part of *Stichopus japonicus* by means of paper chromatography, there were found: (1) aspartic acid (2) glutamic acid (3) glycine (4) alanine (5) valine (6) phenylalanine (7) leucine (8) serine (9) arginine (10) lysine (11) cystine (12) tyrosine (13) arcaine (14) threonine (15) betaine (16) ornithine and (17) adenine, etc. That is to say, the chemical components found in both the meat and skin parts are almost the same.

Among those chemical components, tyrosine was again found in the two-dimentional paper chromatograms of the extracts of meat and skin parts respectively, of which the first run was with 1N ammonium saturated butanol and second run with phenol containing 10% water, and developed spots were tested by Pauli reaction. In those chromatograms, the spot of histidine was not confirmed by ninhydrin and p-bromaniline.

Arginine and arcaine also were again confirmed in the two-dimensional paper chromatograms of the extract of both meat and skin parts of which the first run was made with a mixed solution of butanol, acetic acid, pyridine and water (4 : 1 : 1 : 2) and second run with 10% ammonium saturated butanol. The developed spots were tested by the Sakaguchi reaction.

Tyrosine, phenylalanine also were again confirmed in the two-dimensional paper chromatograms of the extractives of both parts of which the first run was carried out with a mixture of lutidine and aniline, the second run with phenol containing 10% water, and the adsorbed spots were tested by Xanthoprotein reaction.

Glycine, alanine, betaine, arginine, adenine which have been previously found, and creatinine newly were confirmed in the extract of meat part in the two-dimensional paper

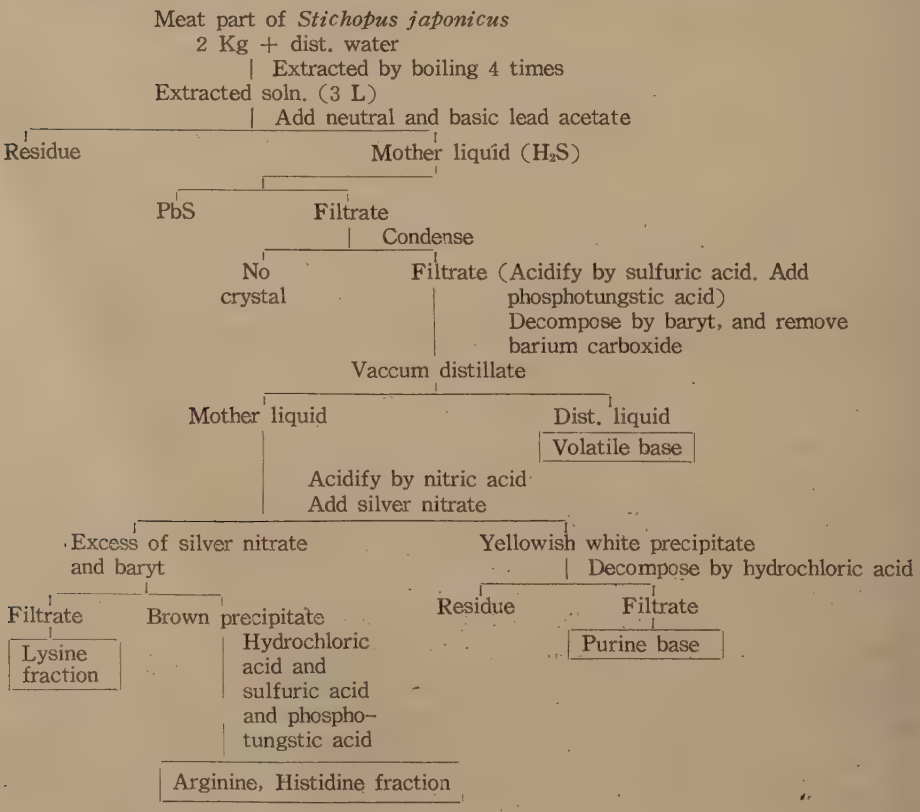
chromatogram of which the first run was made with a mixture of n-butanol, ethanol and water (4 : 1 : 2) and second run with a mixture of n-butanol, acetic acid and water (4 : 1 : 2). The developed spots were tested by Dragendorf's reagent. Creatinine was found in a partition chromatogram of which the spots were tested by Jaffé's reaction. But creatine was not confirmed.

From the results obtained as described above aspartic acid, glutamic acid, glycine, alanine, valine, phenylalanine, leucine, serine, arginine, lysine, cystine, tyrosine, arcaine, threonine, betaine, ornithine, adenine, creatinine were found to exist in the meat and skin parts of *Stichopus japonicus*. Those components are almost the same as those in the extracts of fish meat.

3. Chemical components in the basic fraction

In the previous article 2 of this paper, chemical components in the extractive of

Scheme 1. Fractionation of the extract of meat part of *Stichopus japonicus*



meat and skin parts of *Stichopus japonicus* were detected by two-dimensional paper chromatography, but it was difficult to make a full analysis of all the spots adsorbed in the filter paper. Therefore a determination of the basic fraction was separately undertaken in the following manner. The sample was analyzed by two dimensional paper chromatography.

(1) Preparation of sample

The extract of meat part of sea cucumber was fractionated in the following manner.

(2) Experimental results

Chemical components in the volatile base fraction were ascertained by partition paper chromatography and visible maps were treated with ninhydrin and Dragendorf's reagents, but the Rf values of the maps were not determined. Chemical components in purine base fraction were determined by partition paper chromatography of which the first run was made with butanol acetic acid and adsorbed spots were tested by nitrate solution containing 0.25 M mercury nitrate. Adenine was detected, but hypoxanthine, xanthine, guanine were not found.

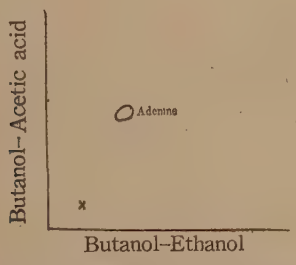


Fig. 3. Paper chromatograms showing purine base fraction

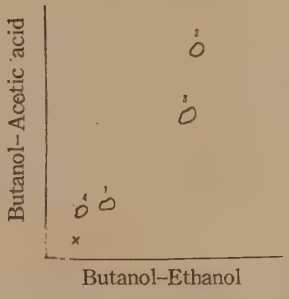


Fig. 4. Paper chromatograms showing arginine and histidine fraction

1. Arginine 2. Arcaine 3. Creatinine 4. Ornithine

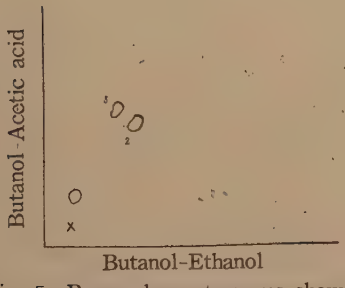


Fig. 5. Paper chromatograms showing lysine fraction

1. Lysine 2. Betaine 3. Choline

In the arginine and histidine fraction, methylguanidine, which is often found in animal bodies, was not detected by the ninhydrin reaction, Sakaguchi reaction nor Jaffé's reaction in the same chromatography.

In the lysine fraction, choline was detected with Dragendorf's reagent and ninhydrin reaction by the same chromatography.

A map in the chromatogram in the fraction of volatile base which is positive for Dragendorf's reagent is supposed to

represent trimethylamine, because choline, glycine and betaine were clearly detected as above described. But the point is questionable.

As one may see from the above described results, adenine, arginine, arcaine, creatinine, ornithine, choline, betaine, lysine were detected in the fraction of volatile base.

Summary

The nitrogen distribution of the extracts of meat and skin parts of *Stichopus japonicus* is inferior quantitatively to that of fish meat. The absolute amount of total nitrogen in the extract of *Stichopus japonicus* is inferior to that of fish meat. Consequently the meat of *Stichopus japonicus* is light in taste.

The sorts of chemical components of extract of meat and skin parts of *Stichopus japonicus* were detected to be as follows: aspartic acid, glutamic acid, glycine, alanine, valine, phenylalanine, leucine, serine, arginine, lysine, cystine, tyrosine, arcaine, threonine, betaine, ornithine, adenine, creatinine, choline.

Those components are almost the same as those of fish meat.

Literature cited

1) Simidu, W. (1948). *Bull. Jap. Soc. Sci. Fish.* **15** (1), 35.
2) Yoshimura, K. & Kubo, S. (1952). *Bull. Fac. Fish., Hokkaido Univ.* **3** (3), 205.
3) Simidu, W. *et al.* (1953). *Bull. Jap. Soc. Sci. Fish.* **19** (8), 871.
4) Tanikawa, E., Akiba, M. & Yoshitani, S. (1955). *Bull. Fac. Fish., Hokkaido Univ.* **5** (4), 341.

STUDIES ON THE NUTRITIVE VALUE OF THE MEAT OF SEA CUCUMBER (*STICHOPUS JAPONICUS* SELENKA)

VII. Inorganic Matter in the Meat of *Stichopus japonicus*

Eiichi TANIKAWA and Tetsuro WAKASA
Faculty of Fisheries, Hokkaido University

To determine the nutritive value of *Stichopus japonicus*, the kind and quantities of inorganic matters must be considered, also the quality of the nutrient (complete protein or not), its quantity (calories of food) and the availability of the food (digestibility).

The present authors have undertaken to study the kind of inorganic matters which exist in the so-called meat of *Stichopus japonicus* which is different from fish meat in many respects.

(1) Preparation of samples

Bodies of *Stichopus japonicus* were eviscerated and the meat part was cut and crushed homogeneously. Fifty grams of crushed meat (water-content 89.2%) was put into decomposing glass bottle and then a small amount of conc. HNO_3 was added. The mixture was decomposed on low fire with supplement of conc. HNO_3 little by little for 4～5 days. When the heating solution became transparent, the decomposition stopped. The decomposed solution was diluted to 100 cc and then was employed for the estimation of the amounts of the various sort of inorganic matter. For the estimation of chlorine, skinned meat was employed directly.

(2) Analysis of inorganic compounds

(i) Ash : By usual method.

(ii) HNO_3-insoluble matter : The filtered residue was dried with filter paper, heated to ash and weighed.

(iii) Sulfuric acid (SO_3'') : The amount of sulfuric acid was estimated as $BaSO_4$ by usual method.[1]

(iv) Phosphoric acid (P_2O_5) : The amount of P_2O_5 was estimated as $Mg_2P_2O_7$ by usual method.

(v) Chlorine : By Mohr's method.[1]

(vi) Calcium : By usual method.[2]

(vii) Magnesium : By usual method.[2]

(viii) Potassium : By Suzuki's method.[3]

(ix) Iron : By usual method.[4]

(x) Copper : By jodometry.[5]

(3) Experimental results

The results obtained are shown as Table 1. In this experiment, the amount of

aluminium was not estimated. But Ōya and Shimada[6] have determined by analysis that aluminium in the meat of *Stichopus japonicus* is about 4.4 mg%.

Table 1. Mineral composition of *Stichopus japonicus* meat

Items	mg% (in original)	mg% (in dry matter)
Nitric acid insoluble material (as oxide)	31.0	208.7
SO_3	298.0	2760
P_2O_5	179.0	1660
Cl	87.0	806
Ca	3.0	27.8
Mg	0.36	3.34
K	23.0	213
Fe	8.2	76
Cu	2.8	25.9
Ash	820.0	7600

Comparing the amounts of inorganic matters in the meat part of *Stichopus japonicus* with those in fish meat, the amount of P_2O_5 of meat part of *Stichopus japonicus* is 1660 mg (in the dried matter), that is to say, the amount of P is about 363 mg%. On the other hand, the amount of P of fish meat,[6] sardine, carp, grey mullet, mackerel, bonito, was 800～7200 mg% (water-content average 75%). The amount of P in the meat of *Stichopus japonicus* is smaller than that in fish meat. The amount of K in fish meat is 80～500 mg% (in the dried matter); on the other hand, the amount of this component in the meat of *Stichopus japonicus* is 213 mg%. That is to say, the amount of K in the meat of *Stichopus japonicus* is about half the value of the fish meat.

The amounts of calcium and magnesium in the fish meat are 100 mg% and 50～100 mg% respectively. The amounts of those components in the meat part of *Stichopus japonicus* are 27.8 mg% and 3.34 mg% respectively. The amounts of the latter are smaller than those of the former. In the meat of the land animals, the amount of magnesium is larger than that of calcium. On the other hand, the amount of calcium in fish is larger than that of magnesium. In the meat of *Stichopus japonicus* also, the amount of calcium is larger than that of magnesium.

The amount of iron in the meat of *Stichopus japonicus* is 76 mg% (in the dried matter), on the other hand, that of fish meat is 6～50 mg%. That is to say, the amount of iron of the meat of *Stichopus japonicus* is larger than that of fish meat. However, the amount of this component of *Stichopus japonicus* is smaller than that of clam (170～200 mg%). Ōya and Shimada[6] have estimated the amount of iron in the meat of *Stichopus japonicus* and stated that it was 74.1 mg% (in the dreid matter).

The amount of copper in the meat of *Stichopus japonicus* was 25.9 mg%. On the other hand, the amount of that component is 0.6–3 mg% (in the dried matter) in fish

meat and 24.6 mg% in oyster meat which is said to be rich in copper-content. That is to say, the amount of copper in the meat of *Stichopus japonicus* is equal to that in oyster meat.

Summary

The amounts of phosphoric acid, calcium, magnesium, potassium of the meat of *Stichopus japonicus* are inferior quantitatively to those of fish meat. But, contrariwise the amounts of iron and copper are larger. The amount of aluminium was not estimated, but it is said by Ōya and Shimada to be larger.

Literature cited

1) Oshima, K., Sasaki, M. & Satodate, K. (1952). *Suisan Jikkenho*. 343 p. Maruzen. (in Japanese).
2) Miyake, Y. & Matsui, H. (1943). *Mizuno Kagaku bunsekiho*. (in Japanese).
3) Suzuki, N. (1952). *Bull. Fac. Fish., Hokkaido Univ.* 3 (1), 62.
4) Suido Kyokai. (1950) *Inryosui no Hantei Hyojun to sono Shikenho*. 280 p.(in Japanese).
5) Ōtsuka, Y. (1945). *Kihon Kagaku Bunsekiho*. 578 p. Kyogoku Shoten. (in Japanese).
6) Ōya, T. & Shimada, K. (1933). *Bull. Jap. Soc. Sci. Fish.* 2 (1), 20-22.

STUDIES ON POST-MORTEM CHANGES IN THE CHEMICAL CONSTITUTION OF THE MEAT OF SEA CUCUMBER (*STICHOPUS JAPONICUS* SELENKA)*

I. Changes in the Amounts of Lactic Acid and Glycogen in the Meat of *Stichopus japonicus* during the Period of Rigor Mortis

Eiichi TANIKAWA, Minoru AKIBA, Hirotoshi ISHIKO and Setsuko YOSHITANI
Faculty of Fisheries, Hokkaido University

When *Stichopus japonicus* is processed into dried merchandise or canned food, it is very important that the raw material of *Stichopus japonicus* must be prepared before the freshness of the meat falls. However, owing to the conditions of fishing it may happen that the raw material of *Stichopus japonicus* can not be treated in fresh state. In this case, the post-mortem changes of *Stichopus japonicus* should be known.

There has been hitherto no published study on such a subject. As to the post-mortem changes of *Stichopus japonicus*, the authors have tried to investigate the changes in rigor mortis, autolysis and putrefaction of the so-called "meat."

The rigor mortis which hardens the muscle of animals is also found to occur in the body of *Stichopus japonicus*. Investigation has been made of the change in the amounts of lactic acid and glycogen in the meat of *Stichopus japonicus* during the period of rigor mortis.

Experimental

1. The change of the hardness of the meat of *Stichopus japonicus* after death

Tauchi[1] tried to judge the freshness of fish meat by his hardometer on the basis of the fact that the fish body gradually becomes soft after the rigor mortis. But his hardometer has a defect of yielding various data even on the same part of the fish body. Tanikawa & Konno[2] have previously tried to judge the freshness of squid meat using Konno's fatiguesmeter of muscles of sportsmen and they succeeded in determination of the freshness.

The present authors have also tried to judge the freshness of the meat of *Stichopus japonicus* by the same instrument in this experiment.

(1) Sample

Living bodies of *Stichopus japonicus* which were caught in the sea near Hakodate were eviscerated and left at room temperature (about 10°C) and the hardness of the bodies was estimated. In this case, the time at which the bodies were eviscerated was supposed to be the time of the death of *Stichopus japonicus*.

---

* This "Studies on post-mortem changes in the chemical constitution of the meat of sea cucumber" is the second part of "Chemical studies on the meat of sea cucumber (*Stichopus japonicus SELENKA*)."

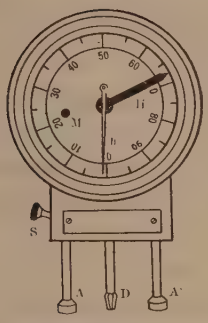


Fig. 1. Fatiguesmeter (Muscle hardness test meter)

(2) Experimental method

To judge the freshness of the meat of *Stichopus japonicus*, the fatiguesmeter of muscles of sportsmen as shown in Fig. 1 was used.

This fatiguesmeter resembles Tauchi's hardometer. In Fig. 1, this meter has 3 feet, A, A' and D, of which A and A' have the same action. D has a weaker spring than A and A'. The needle H is the main needle and the needle h is a sub-needle. M is notice board, connected with S. M and S move by the action of D. Scale is divided into 90, and is read to decimal order. This instrument is used for the determination of the fatigue of human muscles by estimation of the difference of hardness between the strained muscle and the relaxed state of the fatigued muscle by the setting of this meter on the muscle. The needles A and A' are pushed into the loosened muscle to a definite depth, that is to say, when the definite power is applied to the needle D, the switch S indicates "off", with a clicking noise. At the same time, the board changes to red at part M. The scale which is indicated by the needles, H and h, is read. After an estimation, when the switch S is turned back, the red board returns to the white board at M, and the needle H returns to zero. Next, the needles A and A' are pushed into the consciously strained muscle, and so on.

The difference of the values of hardness of strained and relaxed muscles indicates the elasticity of the muscle. The loosened meat of *Stichopus japonicus* was estimated by laying down the body of *Stichopus japonicus* on the table, taking care that the end part of the needles of the instrument should not face to the papillae on the surface. The strained condition of the meat was brought about by laying down the body on a triangular pillow and bending it.

Estimations were made five times and the average value was taken.

(3) Experimental results

The results obtained are shown in Fig. 2. As seen there, the body of *Stichopus japonicus* became hard from the 4th hour after the death until the 44th hour, that is to say, there is a period during which the meat of *Stichopus japonicus* is hard.

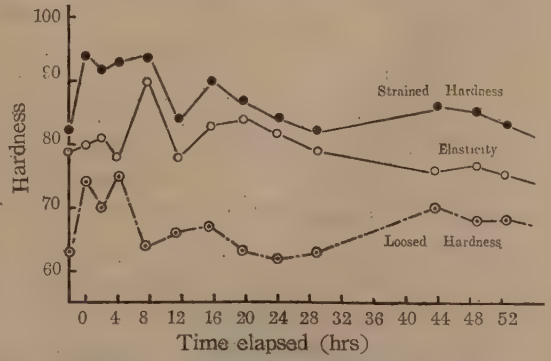


Fig. 2. Changes in the hardness of meat of *Stichopus japonicus* at room temperature (about 10°C)

This phenomenon that the meat becomes hard during such a period may be called "rigor mortis".

2. The post-mortem changes in the amounts of lactic acid, and glycogen in the meat of *Stichopus japonicus*

It is of interest to know the changes in the amounts of lactic acid and glycogen and values of pH in the meat of *Stichopus japonicus* during rigor mortis. The experiment was divided into two parts.

(1) Sample

For the first experiment living *Stichopus japonicus* which was caught in the sea near Hakodate in June was eviscerated and crushed and then left at the room temperarure of 10°C. During the leaving of the crushed meat the changes in amounts of lactic acid and glycogen were observed. For the second experiment, living *Stichopus japonicus* which was caught in July was treated in the same manner. To the material were added each 5 cc of toluene and chloroform. The material was left at room temperature of 15°-18°C; changes of the same items were estimated.

(2) Experimental method

Estimation of the amount of lactic acid: To the crushed meat was added 10 cc of 20% trichloracetic acid to remove the protein. After filtration, the amount of lactic acid in a definite volume of the filtrate was estimated by Friedmann's method.[3]

Estimation of the amount of glycogen: The crushed meat was previously treated by Simonovits' method[4] and the amount of glycogen was estimated by Hagedorn-Jensen's method.[5]

The estimation of the value of pH: The value of pH was estimated by the glass electrode meter.

(3) Experimental results

Experimental results obtained are shown in Fig. 3.

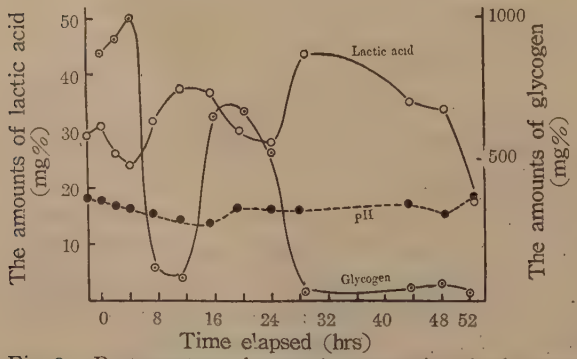


Fig. 3. Post-mortem changes in non-antiseptized meat of *Stichopus japonicus* at 10°C

As seen in Fig. 3, the values of pH of the meat of *Stichopus japonicus* decreased once gradually with the lapse of time after the death, and increased again from the 16th hour after death. Thereafter the value became constant for some hours and increased again from the 50th hour.

The variation of the values of pH has corelated with the variations of the amount of lactic acid. The amount of lactic acid increased after the death. It indicated maximum at the 12th hour, decreased gradually to the minimum amount at the 24th hour, and then increased again until the 30th hour. Thereafter the amount decreased. The amount of glycogen content varied with the increasing and decreasing of the amounts of lactic acid inversely. The rigor mortis of the body of *Stichopus japonicus* continued from about the 4th hour until 44th according to the degree of hardness as previously noted.

In these experimental results, the variation of the amounts of lactic acid and glycogen, when graphed, showed some peaks and valleys.

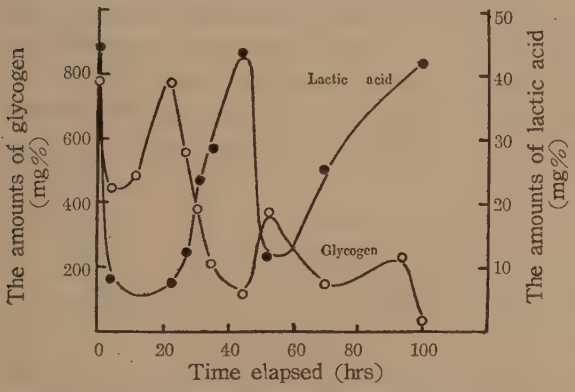


Fig. 4. Post-mortem changes in antiseptized meat of *Stichopus japonicus* at 15°–18°C

In the second experiment, the crushed meat was antiseptized by the addition of toluene and chloroform and the variation in the amounts of lactic acid and glycogen were observed to ascertain whether the results are similar to the first experimental results, or not. The second experimental results obtained are shown in Fig. 4.

As seen in Fig. 4, the amounts of lactic acid and glycogen showed some peaks and valleys of increase and decrease like the first experimental results. But in the second experimental results, as bacterial action in the crushed meat is inhibited by the antiseptants added, the number of mountains and valleys is smaller than in the first experimental results. Further, as the temperature of the first experiment is higher than the second, the position of the mountains in the latter experiment situated to right. In the first experiment, at the time when the increase of the amount of lactic acid stopped, the smell of ammonium began to be noticeable.

Some observations on the results obtained may be worth consideration. The increase of the amount of lactic acid in the meat of *Stichopus japonicus* during the period of rigor mortis is considered to be due to the decomposition of glycogen.

The amount of lactic acid in the meat of *Stichopus japonicus* did not increase slowly, but rapidly for a short time, then decreased and again increased after some interval. The same observation was made by Yamada[6] of variation in the increase of lactic acid in fish meat during the period of the post-mortem change. The phenomenon of decrease and increase of lactic acid during post-mortem change in fish meat is considered to be due to the formation of pyroracemic acid from lactic acid by the action

of lactic acid dehydrogenase, also to be due to the decrease of the amount of lactic acid by the intracellular respiraton remaining after the death of fish.

In the present experiment, the amount of pyroracemic acid and the amounts of various types of phosphoric acid were not estimated, so the variation in the amount of lactic acid can not be explained clearly by the above mentioned chemical changes.

The senior author has observed histologically the meat of *Stichopus japonicus* and ascertained that the edible part of *Stichopus japonicus*, the body wall, is really connective tissue consisting of a network of collagen fiber.[7] The fact that water soluble protein, NaCl solution soluble protein and dil. NaOH solution-soluble protein are fractionated from the body wall, suggests that various kinds of soluble protein may be considered to be bound with a large amount of water (90% of the weight of the body) in that network of collagen fiber in the connective tissue. From the characteristics of the construction of the tissue, the glycolytic action of *Stichopus japonicus* meat is considered differ instinctively from that of fish meat. As to this point, further detailed investigation should be made.

Summary

(1) The variation of the hardness of the meat of *Stichopus japonicus* after death was examined, and the rigor mortis which is seen in fish meat was likewise observed in the "sea cucumber" body.

(2) The variation of the increase and the decrease of the amounts of lactic acid and glycogen was observed to be converse. Those variations are complex, but it is clear that they indicate the phenomenon of the glycolytic action.

Literature cited

1) Tauchi, M. (1931). *Suisan-koshujo-hokoku* 26.
2) Tanikawa, E. *et al.* (1953). *Bull. Fac. Fish., Hokkaido Univ.* 4 (3), 224-233.
3) Friedmann, T. E. *et al.* (1927). *J. Biol. Chem.* 73, 335.
4) Simonovits. (1933). *Biochem. Z.* 265, 437.
5) Hagedorn, H. C. & Jensen, B. N. (1923). *Ibid.* 135, 47.
6) Yamada, K. *et al.* (1948). *Bull. Jap. Soc. Sci. Fish.* 14 (1), 23-26; 41-47.
7) Tanikawa, E. (Unpublished).

STUDIES ON POST-MORTEM CHANGES IN THE CHEMICAL CONSTITUTION OF THE MEAT OF SEA CUCUMBER (*STICHOPUS JAPONICUS* SELENKA)

II. Autolysis of the Meat of *Stichopus japonicus*

Eiichi TANIKAWA, Minoru AKIBA and Setsuko YOSHITANI
Faculty of Fisheries, Hokkaido University

There is no published study on the autolysis of the meat of *Stichopus japonicus*. The so-called "meat" (edible part) of sea cucumber is not true muscular tissue histologically, but is mainly connective tissue.[1] Therefore there may perhaps be some differences between the so-called "meat" and fish meat muscle.

The authors undertook to investigate the autolytic phenomena when the bodies of *Stichopus japonicus* are left in the air. They have determined the optimum pH for autolysis, and have estimated the periodical changes of the amount of autolyzed products in the solutions of various pH values, and velocity of the autolysis of the meat of *Stichopus japonicus* at various temperatures.

1. Phenomena of autolysis when the bodies of *Stichopus japonicus* are left in the air

After the death of *Stichopus japonicus*, and while the body is left in the air, the meat is observed to soften gradually with the lapse of the time and to become mucous matter.

When the body of *Stichopus japonicus* is left on straw, the phenomena of autolysis occur rapidly; when the body is bound up with straw rope, the body is divided into two parts from the point of the binding. It is said that those phenomena are due to the action of some enzymes in the straw, but that point remains yet unknown.

The authors have observed the degrees of the phenomena of autolysis in the appearance of the bodies of *Stichopus japonicus* under various conditions of leaving.

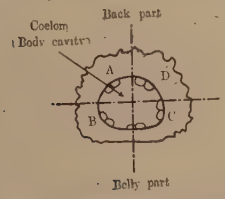


Fig. 1. Eviscerated *Stichopus japonicus* preparation

(1) Samples

As samples, the bodies of *Stichopus japonicus* were left as they were and eviscerated bodies were also employed. The eviscerated body was quartered as shown in Fig. 1.

(2) Experimental method

Each piece of the eviscerated body and non-eviscerated body was left on an enamelled iron square dish or on the straw, in the ice box ($10°\pm4°C$) and in the room ($20°\pm4°C$) respectively.

Those pieces so left were taken up at definite hour intervals, and the mucous matter which had formed on the surface of the sample was removed carefully by pincette. Then the residual solids were weighed. The percentage of the difference in the weight of the

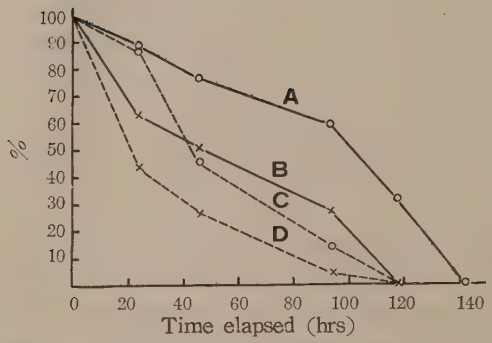


Fig. 2. Auto-viscidation of eviscerated meat

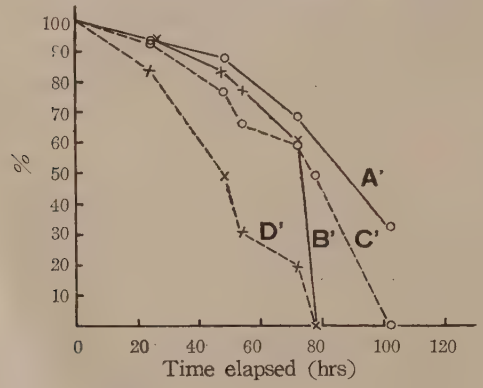


Fig. 3. Auto-viscidation of non-eviscerated meat

piece before leaving and the weight of the piece from which the mucous matter was removed was calculated for the weight of the piece before having been left.

(3) Experimental results

The results obtained are shown in Figs. 2 and 3. Table 1 shows the difference (%) of the degree of autolytic phenomena of *Stichopus japonicus* at various temperatures.

As seen in Table 1, samples C, D (eviscerated quarter piece left in the room) and C′, D′ (non-eviscerated body left in the ice box) show larger degree of autolysis than samples A, B (eviscerated quarter piece left in the room) and A′, B′ (non-eviscerated body left in the ice box).

Samples left at the room temperature evidence a larger degree of autolysis than samples left at ice box temperature. That is to say, the samples at higher temperature are autolyzed more rapidly than those at lower temperature.

Then, whether the pieces of the body of *Stichopus japonicus* which were left on the straw have more rapid autolysis than pieces which were not so left was observed, and results are shown in Table 2.

Table 1. Differences in the degree of auto-viscidation of *Stichopus japonicus* meat stored at various temperatures (%)

Time (hrs) / Differences	0	24	46	48	54	72	78	94	102	118	142
I Sample (C-A)	0	2.15	31.29	—	—	—	—	45.15	—	31.22	—
II Sample (D-B)	0	19.46	24.10	—	—	—	—	24.28	—	—	—
III Sample (C′-A′)	0	1.00	—	10.84	13.59	7.24	9.36	—	32.63	—	—
IV Sample (D′-B′)	0	8.89	—	34.16	45.69	40.86	—	—	—	—	—

Table 2. Differences in the degree of auto-viscidation of *Stichopus japonicus* meat stored on straw mat or iron square dish (%)

Differences \ Time(hrs)	0	24	46	48	54	72	78	94	102	118	142
I Sample (A-B)	0	25.74	25.82	—	—	—	—	31.94	—	31.22	—
II Sample (C-D)	0	43.05	18.63	—	—	—	—	10.07	—	—	—
III Sample (A′-B′)	0	0.39	—	4.38	2.97	7.45	58.28	—	—	—	—
IV Sample (C′-D′)	0	8.28	—	27.70	35.07	30.06	48.92	—	—	—	—

As seen in Table 2, the piece left on the straw shows more rapid autolysis than that on the enamelled iron square dish. The factor of leaving of the piece on the straw exerts greater influence than the factors of temperature and treatment. More explicitly, there is no difference of the degree of autolysis between eviscerated and non-eviscerated bodies, or between the bodies left at room temperature and at ice box temperatures. Comparing the time in which the entire bodies of *Stichopus japonicus* become mucous matter as a result of autolysis, the eviscerated sample requires longer time than the non-eviscerated sample.

2. Relation between the autolysis of the meat of *Stichopus japonicus* and hydrogen ion concentration

According to Ōya[2], in the autolysis of fish meat, the addition of acid stimulates the velocity of the autolytic activity, but the addition of alkali inhibits autolysis. The autolytic activity of fish meat shows generally the most active at about pH 4.5.

At this value of pH, the increase in the amounts of soluble nitrogen, polypeptide nitrogen and amino acid nitrogen are the largest.

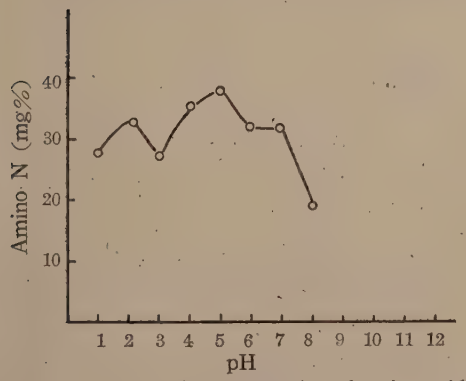


Fig. 4. Changes in the amount of amino acid nitrogen in the solutions adjusted to various pH values

In order to learn the optimum pH of the meat of *Stichopus japonicus*, the following experiments were made.

(1) Sample

Bodies of *Stichopus japonicus* which were caught in October in the sea near Hakodate were eviscerated and skinned as reported previously.[7] The meat part was crushed and employed as the sample.

As seen in Fig. 4, the amounts of amino acid nitrogen shows two peaks pH 2.2 and pH 4.8. In any case, the

the autolysis of the meat of various kinds of fish[2], and he has observed that the optimum pH of fish meat autolysis was generally near 4.5*. Explaining a higher amino acid values obtained in 2.2 of the pH value, according to the present author, the tissue of *Stichopus japonicus* meat are considered to contain various protein components in the network of collagen fiber, differing from the tissue of fish meat[1]. There seems to be some relation between the histological characteristic and the chemical behavior in pH 2.2 of the meat of *Stichopus japonicus*. For example, according to Takahashi,[4] collagen fiber shows the maximum swelling at near pH 2 in the experiment on the skin of fish. The maximum swelling of the meat of *Stichopus japonicus* is considered to occur at and also to be decomposable at near pH 2. There also seems to be some relation between the chemical behavior in pH 2.2 of the decomposition and the isoelectric point of the meat. For example, the isoelectric point of the meat of *Stichopus japonicus* is one-sided being more acidic than that of fish meat—the isoelectric point of the meat of *Stichopus japonicus* is pH 4.2, the Atka mackerel meat is pH 5.2,[5] and the horse mackerel is pH 5.6[6].

Summary

(1) After the period of rigor mortis is passed, the autolysis was observed also in the meat of *Stichopus japonicus*.

(2) The optimum pH of the autolysis of the meat of *Stichopus japonicus* was considered to be near 4.8.

(3) The amount of the decomposition product reached equilibrium at pH 4.5 at 20 hours after leaving.

(4) In the range of experimental temperatures (3°～30°C), the higher the temperature is, the more active is the autolysis. This result is generally similar to what is observed in the case of fish meat.

Literature cited

1) Tanikawa, E. (Unpublished).
2) Ōya, T. (1928). *Suisan gaku kaiho* **5** (1), 1.
3) A. O. A. C. (1935). Chap. XXVIII, 360. 4th Ed. Association of official agricultural chemists.
4) Takahashi, T. (1954). *Bull. Jap. Soc. Sci. Fish.* **19**(12), 1200-1218.
5) Fujii, Y. (1954). *Bull. Fac. Fish., Hokkaido Univ.* **5**(3), 253-276.
6) Okada, M. & Tada, S. (1953). *Bull. Jap. Soc. Sci. Fish.* **19**(3), 178-184.
7) Tanikawa, E., Akiba, M. & Yoshitani, S. (1955). *Bull. Fac. Fish., Hokkaido Univ.* **5** (4), 341.

* Ōya has examined the optimum pH for the autolysis of fish meat by using fish meat suspension. On the contrary, the present authors have employed raw crushed meat. So the results which were obtained by the former and by the latter can not be compared mutually.

STUDIES ON POST-MORTEM CHANGES IN THE CHEMICAL CONSTITUTION OF THE MEAT OF SEA CUCUMBER (*STICHOPUS JAPONICUS* SELENKA)

III. The Putrefaction of the Meat of *Stichopus japonicus*

Eiichi TANIKAWA, Terushige MOTOHIRO and Tetsuro WAKASA
Faculty of Fisheries, Hokkaido University

The chemical components of the meat of *Stichopus japonicus* are different from fish meat muscle in the larger amount of water content and the smaller total amount of nitrogen content. This subject has been treated in an earlier paper, article II.[1] The larger amount of water content in the meat of *Stichopus japonicus* is supposed to have intimate relation with the putrefaction of the meat.

In order to ascertain the type of putrefaction, the velocity of bacterial decomposition was estimated by calculating the velocity constant (the volatile base producing velocity constant), temperature constant and temperature coefficient.

1. Velocity of bacterial decomposition

(1) Sample

Bodies of *Stichopus japonicus*, which were caught in the sea near Hakodate were eviscerated, and crushed. The crushed meat was employed for the experiment.

(2) Experimental method

The crushed meat was put in separate bottles with wide mouths which were plugged with cotton. The material was left in aerobic condition at various temperatures: 16°, 28°, 35° and 45°C (± 2°C respectively). On the other hand, samples of the crushed meat were also left in anaerobic condition at 19° and 35°C respectively. Anaerobic condition was secured by leaving several bottles with sample in a vacuum glass vessel, in which pyrogalic acid and sodium hydroxide were added.

The skin part of *Stichopus japonicus* as shown in Fig. 1 in the previous experiment,[2] was also crushed and left in aerobic condition at 28°C.

The samples were taken up at definite time intervals, and employed for the following various estimations. Determination of pH: By glass electrode meter. Volatile base nitrogen: By Weber and Wilson's method using 10 g of the sample.

(3) Experimental results

Results obtained are shown in Table 1– Table 6 and Fig. 1– Fig. 3.

Table 1. Chemical changes and organoleptic inspection of *Stichopus japonicus* meat during aerobic putrefaction at 16°C

Item \ Time (hrs)	0	20	44	68	115	141
Volatile base-N(mg%)	7.06	6.39	12.92	16.72	35.04	32.36
Amino-N (mg%)	158.8	214.4	259.3	214.4	185.2	92.6
pH	6.1	5.9	6.2	6.25	6.3	6.4
$HgCl_2$-reaction						
A soln.	−	−	−	−	−	−
B soln.	−	−	±	±	±	±
Organoleptic test	freshness fishy smell	〃	slight stale	stale	putrefactive odor	〃

Table 2. In the case of aerobic putrefaction at 28°C

Item / Time (hrs)	Surface skin part		Meat part					
	Volatile base-N (mg%)	pH	Volatile base-N (mg%)	Amino-N	pH	$HgCl_2$-reaction A soln.	$HgCl_2$-reaction B soln.	Organoleptic test
0	8.4	5.8	64	66.6	5.8	−	−	freshness fishy smell
20	15.1	6.2	24.4	56.8	6.0	−	±	
27	15.1	6.5	23.0		6.1	−	±	
42	23.0	6.5		66.6	6.2	−	±	
48	16.6	6.6	15.3		6.2	−	±	
50			38.4	56.4		−	±	putrefactive odor
65			52.8		6.3	−	±	
69	56.3	6.7		51.3		−	±	
72			38.4			−	±	
92	70.3	6.9	105.6	94.8	6.0	−	±	

Table 3. In the case of aerobic putrefaction at 35°C

Item \ Time (hrs)	0	9	23	32	44	71	92
Volatile base-N (mg%)	8.4	19.7	29.7	37.7	70.7	83.4	163.3
pH	6.05		5.7	5.8	5.95	4.9	4.3
Organoleptic test		slight putrefactive odor		strong putrefactive odor			

Table 4. In the case of aerobic putrefaction at 45°C

Item \ Time (hrs)	0	18	24	32	40
Volatile base-N (mg%)	8.4	37.7	41.7	56.3	95.6
pH	6.06	5.85	5.4	5.55	5.8
Organoleptic test		putrefactive odor			

Table 5. In the case of anaerobic putrefaction at 19°C

Time (hrs) \ Item	Volatile base-N (mg%)	pH
0	5.7	6.25
41	19.0	6.0
44	20.3	6.05
48	20.7	5.95
68	25.0	6.0
90	28.4	6.05
119	33.0	6.15
144	45.7	6.2
162	33.7	5.85
167	49.7	6.0

Table 6. In the case of anaerobic putrefaction at 35°C

Time (hrs) \ Item	Volatile base-N (mg%)	pH
0	8.4	6.05
19	17.7	6.1
26	17.7	5.4
38	21.0	5.7
69	41.7	5.15
95	73.1	5.4

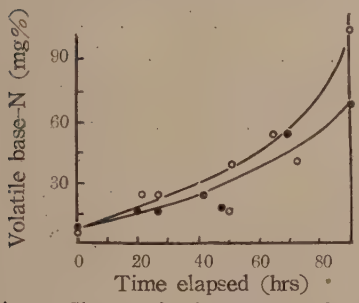


Fig. 1. Changes in the amount of volatile base nitrogen during aerobic putrefaction at 28°C

○——○ Volatile base-N (surface skin part)
●——● Volatile base-N (meat part)

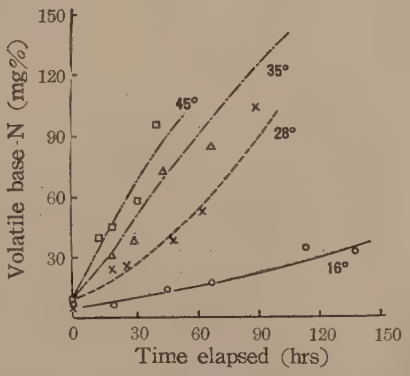


Fig. 2. Changes in the amounts of volatile base nitrogen during aerobic putrefaction at various temperatures

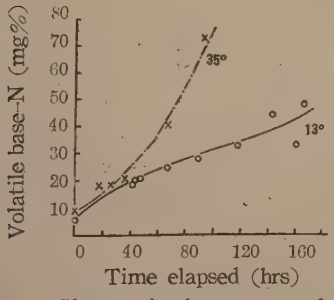


Fig. 3. Changes in the amount of volatile base nitrogen during anaerobic putrefaction at 35° and 13°C

As seen in the Tables and Figs., the higher the leaving temperature, the larger the amount of volatile base nitrogen is. That is to say, the velocity of bacterial decomposition of the meat of *Stichopus japonicus* becomes more rapid with the rising of temperature within the range of present experiment temperatures. The value of pH decreased temporarily with the prolongation of leaving time at temperatures above 35°C, but the value increased without

temporarily decreasing at lower temperatures. In the previous experimental results,[3] the value of pH of the meat of *Stichopus japonicus* in rigor mortis decreased temporarily and then increased again.

In this experiment. if the sample is examined after a short time material, the temporary decreasing of the value of pH may be observed.

As seen in Fig. 1, the meat part seems to be more decomposable than skin part.

From Figs. 2 and 3, the relation between the storing time (hrs) "t" and the values of "log y/A-y" corresponding to the storing time, was plotted as shown in Fig. 4, similarly to what was done in a previous study on crab meat.[4] Here, "y" is the increasing amount of volatile base, "A" is the maximum amount of volatile base nitrogen produced, but the value "A" in this experiment can not be found so the value of "A" was considered to be the value of "y" after the storing time of 40 hrs, e. g. 18 mg% at 16°C, 38 mg% at 28°, 70 mg% at 35°, 90 mg% at 45° aerobically, and 20 mg% at 19°, 70 mg% at 35°C anaerobically, respectively. "t" is time (hrs) from the starting point of the experiment until the estimation of the amount of volatile base nitrogen. The relation between "t" and the values of "log y/A-y" is linear. The value of the bacterial decomposition velocity constant "K" is shown as the degree of the declination of the straight line.

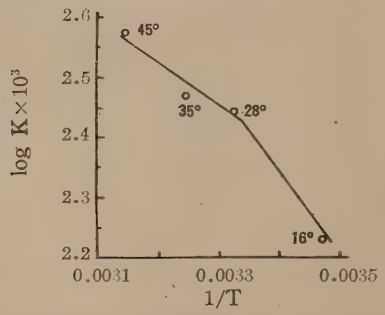


Fig. 4. Relation between the values of "log K×10³" and "1/T"

Here, the values of "K" are shown in Table 7.

Table 7. "K" values of *Stichopus japonicus* meat at various temperatures

Temp. (°C) / Leaving condition	16°	19°	28°	35°	45°
Aerobic	17	—	28	29	37
Anaerobic	—	6	—	34	—

As seen in Table 7, the value of K × 10^3 was 17-37 in the range of 16°-45°C aerobically, and 6 at 19°, 34 at 35°C anaerobically, respectively. Below 30°C, the anaerobic decomposition occurs later than the aerobic decomposition. At higher temperatures, there is no difference between them.

The values of temperature constant (B) and temperature coefficient (Q_{10}) which were calculated from a similar equation to the one used in the previous study on crab meat,[4] are shown in Table 8.

Table 8. "Q_{10}" and "B" values of *Stichopus japonicus* meat

Leaving condition	Temp. (°C)	Q_{10}	B
Aerobic	16 ~ 28°C	1.5	6500
	28 ~ 45°C	1.2	34300
Anaerobic	19 ~ 35°C	3.1	19900

As seen in Table 8, in the aerobic storing the values of "Q_{10}" are 1.2–1.5 in the range of 16°–45°C; in the anaerobic storing the value was 3.1 in the range of 19°–35°C.

From those results, the meat of *Stichopus japonicus* in anaerobic condition is seen to be less decomposable than in aerobic condition, but if the decomposition once starts, the meat in anaerobic storing is more decomposable than in aerobic storing corresponding to the difference of storing temperature.

The values of the bacterial decomposition velocity constant "K", temperature constant (B), and temperature coefficient (Q_{10}) of the meat of *Stichopus japonicus* are compared with corresponding values for other fish, crab and squid meat, e. g. crab meat (*Erimacrus isenbeckii*), Atka mackerel (*Plourogrammus azous*),[5] mackerel (*Scomber japonicus*),[6] Saury (*Cololabis saira*),[7] squid (*Ommastrephes sloani pacificus*),[8] caught in autumn, which were investigated previously by the senior author.

The respective values for comparison are shown in Table 9 and Table 10.

As seen in Tables 9 and 10, the bacterial decomposition velocity constant "K", of squid meat is the largest among them, mackerel, crab, Atka mackerel are next to squid meat in that order. The meat of *Stichopus japonicus* is the smallest. That is to say, the meat of *Stichopus japonicus* is less decomposable. This is perhaps owing to the fact that the meat of *Stichopus japonicus* has a small amount of meat extractive and the "meat" consists principally of connective tissue, collagen, as stated lately in "Studies on the proteins of the meat of Sea Cucumber (*Stichopus japonicus SELENKA*)".[9]

Table 9. Comparative value of "K" in *Stichopus japonicus* and other fish meat

Species \ Temp. (°C)	14°–16°	19°–21°	24°–28°	33°–37°	45°±2°
Sea cucumber meat (Aerobic)	17	—	28	29	37
Sea cucumber meat (Anaerobic)	—	6	—	34	—
Crab meat (Aerobic)	—	—	74	91	—
Crab meat (Anaerobic)	—	—	71	—	—
Atka mackerel meat (Aerobic)	—	22	36	60	—
Mackerel (〃)	30	—	99	114	134
Saury (〃)	27	—	30	48	59
Squid (〃)	—	77	103	151	—

Table 10. Comparative values of "Q_{10}" and "B" in *Stichopus japonicus* and other fish meat

Materials	Range of temp.	Q_{10}	B
Sea cucumber meat			
Aerobic	16°~28°	1.5	7×10^3
Aerobic	28°~45°	1.2	34×10^3
Anaerobic	19°~35°	3.1	20×10^3
Crab meat (Aerobic)	4°~35°	1.2	3×10^3
Atka mackerel	over 25°C	1.5	8×10^3
(〃)	below 25°C	3.5	21×10^3
Mackerel	over 25°C	1.2	3×10^3
(〃)	below 25°C	3.6	22×10^3
Saury (〃)	below 40°C	1.3	5×10^3
Squid (Autumn) (〃)	below 40°C	1.6	9×10^3

The values of temperature constant (B) and temperature coefficient (Q_{10}) of mackerel meat are the largest amongst all the meats of fish, crab and squid. Atka mackerel, squid, *Stichopus japonicus*, saury and crab meat are next in order.

2. Increase and decline of the production of organic bases accompanying the bacterial decomposition of the meat of *Stichopus japonicus*

When the meat of *Stichopus japonicus* is stored at 25°C, the amount of volatile base nitrogen incearses with the lapse of time.

During the bacterial decomposition, the organic bases were detected.

(1) Sample

The sample employed in this experiment was the same as in article I.[1)]

(2) Experimental method

The crushed meat was left aerobically at 25°C, and taken up for the experiment.

The estimation of the amount of volatile base nitrogen was done by Weber and Wilson's method. Organic bases were detected by paper chromatography. The preparation of samples, developing solvents, and revealing reagents were as follows.

a) Volatile bases: Each 30 g of the crushed meat which was taken at definite time intervals, was put in a flask. Five cc of 1 N HCl was put in a receiver which was kept to soak in the lowest end of the cooler which was connected with the flask. Five cc of 10 % NaOH was added to the sample and distilled by steam. When 50 cc of the distillate was obtained (after 45 minutes' distillation), the distillation was stopped. The distillate was transferred into an evaporating dish, evaporated to dryness on a water bath, and the excess of HCl was driven off. Then, hydrochloride of volatile bases obtained was diluted with 0.5 cc of water and employed for the chromatography. As solvents a mixture of n-butanol, acetic acid and water (3 : 1 : 2) was employed in one dimensional (ascending) chromatography. Developed spots were revealed by spraying of

0.1 % ninhydrin-n-butanol solution.

b) Non volatile bases: Ten g quantity of the crushed meat was taken and adjusted to below pH 3.0, diluted with 50 cc of 95% alcohol and left for about 2 hours. The precipitated matter was separated by centrifugal separator. As the freshness of the sample falls, the larger becomes the amount of precipitatd matter, and it takes a long time to separate the precipitated matter. After the transparent solution thus obtained was concentrated to 1 cc it was employed for paper chromatography.

The developing reagent was 1 N NH_4OH saturated n-butanol. The spot was developed by ascending method of one dimensional paper chromatograpy. The developed spots were revealed by Diazo reaction. Histidine and tyrosine derivatives were detected. Another spot was developed by a mixture of n butanol, acetic acid, pyridine and water (4 : 1 : 1 : 2), and revealed by Sakaguchi reaction; chemical components containing guanidine radical were detected.

c) Detection of indol and phenols: Fifty grams of the crushed meat was taken and dil. sulfuric acid was added to acidify for the congo-red test paper, and then the solution was distilled out 2/3 of the original volume. Ten grams of NaCl was dissolved in the distillate, and 30 cc of ether was added and shaken. The fraction of indol and phenols is obtained in the ether layer. The ether layer was separated from the water layer. To the separated ether layer sodium sulfate was added to dehydrate. Ether was removed at lower temperature, and the residue was employed for chromatography. As the developing solvent, water saturated n-butanol was used. The spot was developed ascending by one dimensional chromatography. The developed spot of phenols was detected by Millon reaction, and spot of indol was revealed by Ehrlich's reagent (HCl solution of *p*-dimethylaminobenzaldehyde).

(3) Experimental results

The relation between the amount of volatile base nitrogen produced in the meat and the kind of organic bases produced at definite intervals of time is shown in Fig. 5.

In the curve of the production of volatile base nitrogen in the meat which was stored aerobically at 25°C, agmatine in the fraction of volatile bases was detected, also histidine and tyrosine were revealed by Diazo reaction. Arginine and agmatine-like substance were detected at 20 hours' storing (at that time, the amount of volatile base nitrogen was 9.6 mg%). Putrescine and cadaverine in the fraction of volatile base were observed, agmatine was also detected and histidine and tyrosine were detected by Diazo reaction; while arginine, agmatine and arcaine were detected by Sakaguchi reaction, respectively, after 27 hours' storing (at that time, the amount of volatile base nitrogen was 18.5 mg%).

The production of histidine and tyrosine continued to 72 hours' storing after the leaving of the meat (at that time, the amount of volatile base nitrogen was 76.2 mg%).

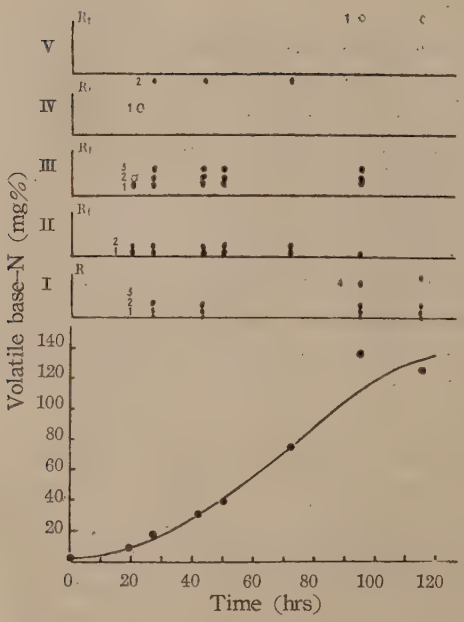


Fig. 5. Changes in the amount of volatile base nitrogen and detected organic bases of *Stichopus japonicus* meat during aerobic deterioration at 25°C

I. Volatile amines
Spot 1. putrescine 2. cadaverine 3. agmatine 4. iso-amylamine (?)
II. Non-volatile amines (Diazo)
Spot 1. histidine 2. tyrosine
III. Non-volatile amines (Sakaguchi)
Spot 1. arginine 2. agmatine 3. arcaine
IV. Tryptophan derivatives
Spot 1. tryptophan 2. Indol
V. Phenol derivatives
Spot 1. cresol

The production of arginine, agmatine and arcaine continued to 95 hours (at that time, the amount of volatile base nitrogen was 139.2 mg%).

A similar spot to that of indol which was revealed after 27 hours' storing, continued to be detectable after 72 hours storing (at that time, the amount of volatile base nitrogen was 76.2 mg%). Spot in the fraction of phenols which was revealed in yellowish green by Millon's reagent after 95 hours' storing and 115 hours' storing seemed to be *p*-cresol (at that time, the amounts of volatile base nitrogen were 139.2 mg% and 125.8 mg% respectively).

Spot in the fraction of volatile bases which was revealed after 95 hours' storing and of which the value of Rf was 0.60, was judged to be iso-amylamine (Rf=0.62) or secondary amylamine(Rf=0.55). It was not ascertained whether the spot is the former or the latter.

Spot in the fraction of volatile bases which was revealed after 115 hours' storing (at that time, the amount of volatile base nitrogen was 125.8 mg%), of which the Rf value was 0.69, seemed perhaps to be not *β*-phenethylamine, but isoamylamnie.

In the authors' experiment on the distribution of amino acid nitrogen in the meat of *Stichopus japonicus*, the amount of nitrogen in the fraction of arginine was estimated to be twice that in other fractions.

In the organic bases produced, the amount of guanidine compounds became larger with the falling of the freshness of the meat, but histamine which is produced from histidine, tyramine which is produced from tyrosine, tryptamine, iso-butylamie, and *β*-phenethylamine were not detected.

Ornithine which is a decomposed interim product from arginine was not detected,

but putrescine which is a decomposed end product was detected, so the presence of ornithine may be admitted.

The presence of agmatine was also detected in the course of the putrefaction of the meat. Both decompositions of arginine perhaps occurred at the same time.

Arcaine which was detected in the meat extractive was also detected in this experiment.

But the amount of arcaine was not ascertained, so it is difficult to judge whether arcaine is a decomposition product or not.

As decomposition products of tyrosine, besides the production of tyramine, the course of production of phenol through oxyphenylacetic acid is expected. In the present experiment, a spot like that of *p*-cresol was also observed. However, it is still open to question whether *p*-cresol or phenol may be produced by bacterial action.

Indol, skatol and indol acetic acid are produced from tryptophan, at the putrefaction of the meat. Although the production of indol was observed, the presence of tryptophan was not detected.

Discussion

Summarising up the results obtained, the process of the decomposition of the meat of *Stichopus japonicus* was similar to that of fish meat in the kind of decomposition products and in the variation of the amount of the products. But the meat of *Stichopus japonicus* is more difficult to decompose than fish meat. This is considered to be due to the small amount of the meat extract, moreover the histological characteristic of the tissue may exert some influence. The meat of *Stichopus japonicus* consists of a collagen fiber network, in which there is contained a large amount of water bound with various kinds of protein.[9]

According to Simidu,[10] there are differences in the decomposition velocity in accordance with the differences of the physical properties (e. g. hardness of the meat). The tissue of the meat of *Stichopus japonicus* which is different histologically from fish meat consists principally of insoluble scleroprotein (structural proteins), therefore, there seems to be difference in the decomposition velocity.

Summary

(1) Aerobic and anaerobic bacterial decomposition of *Stichopus japonicus* meat were discussed in comparison with the same process in fish meat.

(2) It was ascertained that the meat of *Stichopus japonicus* is more difficult to decompose than fish meat. One of the causes of such difference in the velocity of bacterial decomposition was considered to be the histological characteristic.

(3) The variation of the amounts of amino acid and organic bases in the process of the bacterial decomposition was examined, and the results obtained were discussed.

Literature cited

1) Tanikawa, E. *et al.* (1955). *Bull. Fac. Fish., Hokkaido Univ.* **5** (4), 341.
2) ———— *et al.* (1955). *Ibid.*
3) ———— *et al.* (1955). *Ibid.*
4) ———— *et al.* (1953). *Ibid.* **4** (1), 22–31.
5) ———— *et al.* (1954). *Ibid.* **5** (3), 289–298.
6) ———— *et al.* (1954). *Ibid.* **5** (1), 153–163.
7) ———— *et al.* (1954). *Ibid.* **5** (2), 209–221.
8) ———— *et al.* (1954). *Ibid.* **4** (4), 323–336.
9) ———— (Unpublished).
10) Simidu, W. & Hibiki, S. (1954). *Bull. Jap. Soc. Sci. Fish.* **20** (4), 302–304.

STUDIES ON POST-MORTEM CHANGES IN THE CHEMICAL CONSTITUTION OF THE MEAT OF SEA CUCUMBER (*STICHOPUS JAPONICUS* SELENKA)

IV. Determination of the Freshness of the Meat of *Stichopus japonicus* and the Limit of the Freshness for Use as the Raw Material of Canned Food

Eiichi TANIKAWA, Terushige MOTOHIRO and Tetsuro WAKASA
Faculty of Fisheries, Hokkaido University

The meat of *Stichopus japonicus* has been clearly demonstrated to be less decomposable than other fish meat in the previous article III.[1] However, when bodies of *Stichopus japonicus* are used as the raw material of the canned food, it is very important to know the degree of freshness, the limit of the freshness for the raw material and the point of incipient putrefaction.

The authors have tried to throw some light upon those matters by estimating the amount of volatile base nitrogen. Simultaneously, $HgCl_2$-reaction (Amano's method)[2] and also bacterial counts were determined. In view of the previous experimental results, the changes in the freshness, corresponding to the amount of volatile base nitrogen of 20 mg%, were observed in detail.

(1) Sample

Living bodies of *Stichopus japonicus* which were caught in the sea near Hakodate were eviscerated by splitting the ventral side, and were left as they were in flat enamelled iron dishes at $16° \pm 3°C$ and $25° \pm 1°C$ respectively. The freshness of the meat falls with the lapse of the time.

(2) Experimental method

Several bodies which were left as such were taken after the lapse of definite interval hours, and used for the estimation of the amount of volatile base nitrogen, amino acid nitrogen and for the determination of pH values and $HgCl_2$-reaction. For the bacterial counts in the meat, a part of the body remaining was taken in steril condition and crushed in the Waring Blendor, and then was employed.

(i) The amount of volatile base nitrogen was estimated by Weber and Wilson's method.

(ii) The amount of amino acid nitrogen was estimated by Pope and Stevens' method.

(iii) The $HgCl_2$-reaction was determined by Amano's method.

(iv) The pH value was determined by glass electrode meter.

(v) Bacterial count was made by the decimal dilution method.

(3) Experimental results and discussion

The amounts of volatile base nitrogen and amino acid nitrogen at intervals of definite hours' leaving are shown in Tables 1 and 2. In those Tables the values of pH, result

of $HgCl_2$-reaction, bacterial counts, organoleptic observation and kind of the detected organic bases are also recorded.

Figs. 1 and 2 show the changes of the amount of volatile base nitrogen and detected organic bases, and of the bacterial counts of *Stichopus japonicus* meat during the aerobic deterioration at 16°±3°C.

Fig. 3 shows the change of the bacterial counts during the aerobic deterioration at 25°C. The changes in the amounts of volatile base nitrogen and detected organic bases during the deterioration at 25°C have been shown in Fig. 3 in the previous article III.[1]

Table 1 Chemical changes in the meat of *Stichopus japonicus* (16°±3°C)

Time (hrs)	Volatile base-N (mg%)	Amino-N (mg%)	pH	$HgCl_2$-reaction A soln.	$HgCl_2$-reaction B soln.	Bacterial counts	Organoleptic test	Organic bases detected
0	1.9	29.9	6.2	−	±	16×10^5	in rigor mortis	arginine
24	3.2	28.8	5.7	−	±	92×10^5	fresh smell	
48	13.1	27.2	6.4	±	+	27×10^6	end period of rigor mortis, viscous in surface skin,	
58	13.1	43.5	7.0	−	±	20×10^6	fresh smell, edible	
71	14.8	95.2	6.7	−	±	98×10^5	softening, fishy smell, unedible	arginine
76	14.5	57.1	6.6	−	±	27×10^7	good smell, notable viscidation	arginine, indol putrescine
91	18.4	23.0	7.2	−	±	20×10^7	ill smell, 〃 〃	indol
94	20.4	32.6	6.3	−	±		perfect viscidation, muddy stink	arcaine, arginine, putrescine
97	18.4	32.6	6.6	−	±			
99	24.4	29.9	6.8	−	±	60×10^7		
103	30.3	46.2	7.2	−	±			indol, arcaine, agmatine arginine, tyrosine, putrescine

Table 2 Chemical changes in the meat of *Stichopus japonicus* (25°±1°C)

Time (hrs)	Volatile base-N (mg%)	Amino-N (mg%)	pH	$HgCl_2$-reaction A soln.	$HgCl_2$-reaction B soln.	Bacterial counts	Organoleptic test	Organic bases detected
0	3.2	40.8	6.5	±	±	4×10^6	in rigor mortis	
19	9.6	32.6	6.2	−	+	9×10^6	〃 〃, fresh smell	arginine, tyrosine, histidine
27	18.5	35.4	5.8	−	±	10×10^6	no fishy smell, unedible	indol, arcaine, agmatine, tyrosine, histidine, arginine
42	31.7	81.5	5.9	−	±	18×10^6	viscous in surface part, ill smell	〃 〃 〃 〃
50	39.7	34.0	6.4	−	±	12×10^6	notable viscidation, stimulus	arcaine, agmatine, tyrosine, arginine, histidine
72	76.2	35.4	7.0	−	±	20×10^5	discolored in surface part, H_2S smell	indol, tyrosine, histidine
95	139.2	40.8	7.5	±	+	36×10^6	〃 〃	
115	125.8	27.2	8.4	±	+	20×10^6	notable viscous	

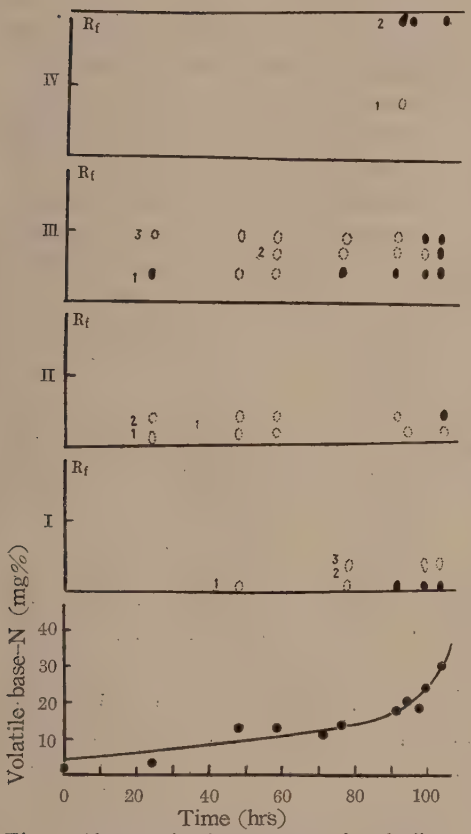


Fig. 1. Changes in the amount of volatile base nitrogen and detected organic bases of *Stichopus japonicus* meat during aerobic deterioration at 16° ±3°C

I. Volatile amines
Spot 1. putrescine 2. cadaverine 3. agmatine
II. Non-volatile amines (Diazo)
Spot 1. hastidine 2. tyrosine
III. Non-volatile amines (Sakaguchi)
Spot 1. arginine 2. agmatine 3. arcaine
IV. Indol fraction (Ehrlich)
Spot 1. tryptophan 2. Indol

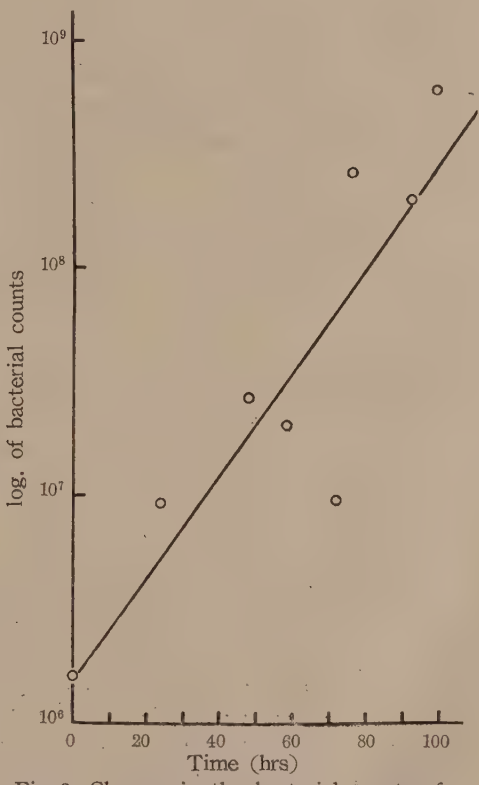


Fig. 2. Changes in the bacterial counts of *Stichopus japonicus* meat during aerobic deterioration at 16° ± 3°C

As seen in Fig. 2, in the case of storing at 16°±3°C, the number of bacteria increased from the initial period of the storing. The number of bacteria amounted to 6×10^8 at 99 hours' storing. After this storing time, the amount of volatile base nitrogen was 24.4 mg%; this storing time corresponds to the initial stage which shows the rapid increasing of the amount of volatile base nitrogen.

In the storing at 25°±1°C, the number of bacteria indicated maximum at 50 hours' storing, thereafter the number did not increase. During this storing time, the amount of volatile base nitrogen increased rapidly following the period of rapid increase in number

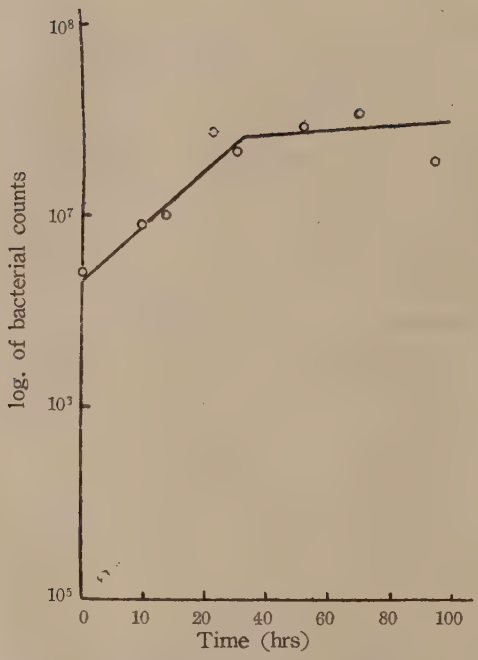


Fig. 3. Changes in the bacterial counts of *Stichopus japonicus* meat during aerobic deterioration at 25°±1°C

of bacteria.

Shewan[3] has studied on the putrefaction of haddock and dogfish, and Fieger & Friloux[4] have studied on the same subject in the case of Gulf shrimp. They have observed the relation between the amounts of volatile base nitrogen or the amino acid nitrogen and the number of bacteria. According to their results, the amount of volatile base nitrogen showed rapid increase after the number of bacteria reached to equilibrium via the lag period.

The authors' result which was obtained in the relation between the amount of volatile base nitrogen and the number of bacteria agreed with the results of Shewan, and Fieger & Friloux. The pH value of the meat decreased temporarily to the acidic side, but it increased in company with the increasing of the amount of volatile base nitrogen at 30 hours' storing. The amount of volatile base nitrogen of the meat increased rapidly after the period of rigor mortis. This is perhaps due to the decomposition by bacteria of amino acids or peptides in free state.

Observing the relation between the amount of volatile base nitrogen and the kind of organic bases detected, it differs dependant upon the storing temperature. Agmatine was detected when the amount of volatile base nitrogen reached to about 9.6 mg% and indol was detected when the amount of volatile base nitrogen reached about 20 mg% (after 90 hours' storing at 16°±3°C or after 27 hours' storing at 25°±1°C.) Putrescine was also detected at the point of production of the amount of volatile base nitrogen of about 20 mg%. Cadaverine-like substance was detected when the amount of volatile base nitrogen reached to 25 mg%.

Observing the $HgCl_2$ reaction of the meat of *Stichopus japonicus*, A-solution indicated (−) and B-solution indicated (±) at the initial period of the storing. When the pH value of meat shifted to the alkali side, A-solution indicated (±), B-solution indicated (+). The $HgCl_2$-reaction of the meat shows contrary to that for fish meat. That is to say, the reaction of the meat extractive of *Stichopus japonicus* is sharp for

B-solution (acidified $HgCl_2$ solution with acetic acid), but it is not sharp for A solution ($HgCl_2$ solution without acetic acid).

That fact that in the fresh meat of *Stichopus japonicus*, B-solution is positive, may be due to the presence of some substance which is stable toward the $HgCl_2$ solution, but is not stable toward acetic acid. If this substance is supposed to be some protein, it may be a mucin which is precipitated by acetic acid.

Taking into consideration the results as above obtained, the mucous matter began to increase on the surface of skin of *Stichopus japonicus* within a short time after rigor mortis, at which time the amount of volatile base nitrogen indicated 10 mg%, and the body was yet edible.

With the decline in the freshness of the meat, at the time when the amount of volatile base nitrogen reached about 20 mg%, indol and putrescine were detected, and the fishy smell of the body changed to a bad smell and the meat became unedible. This point is considered to represent the incipient putrefaction of the meat. The incipient putrefaction of fish meat is at 30 mg% in amount of volatile base nitrogen,[5] but it is better to consider that point of the meat of *Stichopus japonicus* to be at 20 mg% of the volatile base nitrogen, from the results obtained as above stated.

In case *Stichopus japonicus* is prepared for the canned boiled meat, when raw material is used of which the amount of volatile base nitrogen is over 10 mg%, the canned food becomes of bad quality, that is to say, the limit of the freshness of *Stichopus japonicus* for the raw material of canned food is considered to be at about 10 mg% of volatile base nitrogen.

Accordingly, it is important to know the time at which the meat of bodies of *Stichopus japonicus* after death will reach to 10 mg% in the amount of volatile base nitrogen. This time, "t_{10}" is different from the storing temperature. From Figs. 2 and 5 in the previous article III and from Fig. 1 in this article, when the meat is left aerobically at various temperatures, the values of "t_{10}" are as shown below in Table 3 and Fig. 4.

Table 3

Temp. (θ°C)	t_{10} (hrs)
16	45
25	15
28	10
35	4
45	2

According to the results obtained with the meat of crab[6] (*Erimacrus isenbeckii*), saury[7] (*Cololabis saira*), mackerel[8] (*Scomber japonicus*), Atka mackerel[9] (*Pleurogrammus azonus*) and squid[10] (*Ommastrephes sloani pacificus*) by the senior author, the relation between the freshness of the meat and the storing temperatures is shown as the following equation (1).

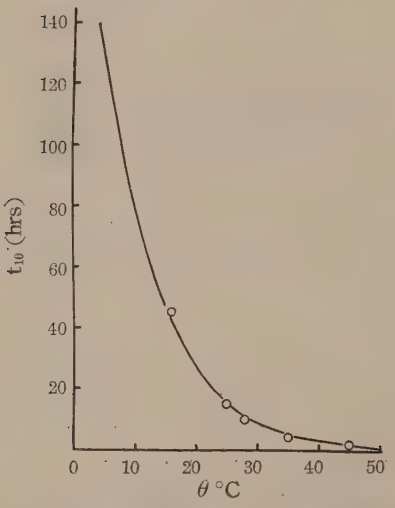


Fig. 4. Relation between the values of "t_{10}" and temperature "t"

$$t_{10\theta} = \alpha - \beta\theta \quad \cdots\cdots\cdots (1)$$

Here, $t_{10\theta}$ is the maximum time in which the freshness of the meat reaches to the limit (10 mg% of the amount of volatile nitrogen) for the raw material at the temperature of θ° C; α and β are the constants.

The relation between the values of "log $t_{10\theta}$" and the storing temperature of "θ° C" is linear as shown in Fig. 5. This straight line is manifested by equation (2).

$$\log t_{10\theta} = 2.322 - 0.0452\theta \cdots\cdots\cdots (2)$$

The relation between the values of $t_{10\theta}$ and the storing temperatures (θ°C) is shown in Fig. 6 as a particular functional scale.

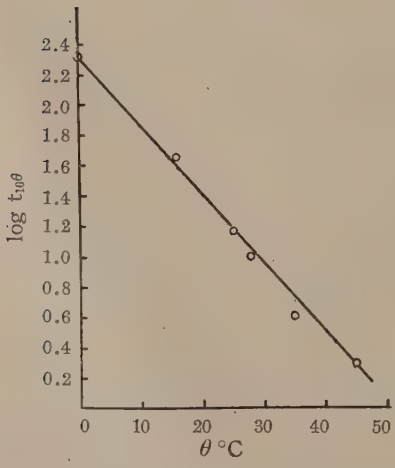


Fig. 5. Relation between the values of "log $t_{10\theta}$" and temperature "t"

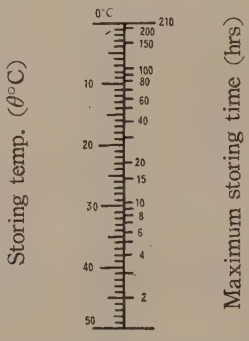


Fig. 6. Functional scale of *Stichopus japonicus* meat to show the relation between the maximum storing time and storing temperature

In Fig. 6, it may be seen that if the room temperature of the cannery is 10°C, the meat of *Stichopus japonicus* must be handled and packed in cans and the sterilization started within 74 hours; if at 20°C, the maximum time is 27 hours; and if at 30°C, the maximum time is 9 hours.

Summary

(1) The change in the freshness of the meat of *Stichopus japonicus* was observed by estimation of the amounts of volatile base nitrogen and amino acid nitrogen, by the $HgCl_2$-reaction (Amano's method), by the values of pH and by the bacterial counts. The estimation of the amount of volatile base nitrogen is considered to be the most suitable for the measurement of the freshness of the meat of *Stichopus japonicus*.

(2) The increase and the decrease of the amount of organic bases were observed in company with the estimation of the amount of volatile base nitrogen.

(3) When the amount of volatile base nitrogen reaches to 20 mg% in the meat of *Stichopus japonicus*, it is considered to be in the state of incipient putrefaction, and the limit of freshness of the raw material safe for use is suggested to be 10 mg% of the amount of volatile base nitrogen.

(4) When the limit of the freshness of meat for edible or for raw material for canned food being decided to be 10 mg% in the amount of volatile base nitrogen, the change of the limit of leaving time of the meat according to the environmental temperatures was examined. The relation between the limit of leaving time and the environmental temperatures has been manifested by a functional scale.

Literature cited

1) Tanikawa, E. *et al.* (1955). *Bull. Fac. Fish., Hokkaido Univ.* **6** (1), 63-72.
2) Amano, K. & Uchiyama, H. (1948). *Bull. Jap. Soc. Sci. Fish.* **14** (1), 48-50.
3) Shewan, J. M. (1951). *Fishing Bull.* **1** (12), 8.
4) Fieger, E. A. & Friloux, J. J. (1954). *Food Tech.* **8** (1), 35-38.
5) Tanikawa, E. (1935). *Suisan-Seizo-Kaishi* **3** (5), 267-296; **3** (6), 319-346.
6) ———— *et al.* (1953). *Bull. Fac. Fish., Hokkaido Univ.* **4** (1), 22-31.
7) ———— *et al.* (1954). *Ibid.* **5** (2), 209-221.
8) ———— *et al.* (1954). *Ibid.* **4** (4), 323-336.
9) ———— *et al.* (1954). *Ibid.* **5** (3), 289-298.
10) ———— *et al.* (1954). *Ibid.* **4** (4), 323-336.

農林産廃液の漁網防腐剤への利用

第1報　亜硫酸パルプ廃液及び硫酸ニコチン製造廃液の硫化処理試験

五十嵐久尙・田ノ上家久
（北海道大学水産学部水産化学教室）

Utilization of Waste Liquors of Agricultural and Forest Products for Net Preservatives

I. Experiment on the sulphuration of waste liquors of sulphite pulp and nicotine sulphate

Hisanao IGARASHI and Iehisa TANOUE

Abstract

Preservatives which were made by the sulphuration of sulphite pulp waste liquor and nicotine sulphate waste liquor, were tested by exposing of cotton lines in sea water. These sulphide dyes showed little superiority, but the sulphite pulp waste liquor mixed with coal tar showed better efficacy and easier drying than the coal tar only for dyeing.

漁網防腐剤として我が国で使用されているものを大別すれば，タンニン剤とコールタールである。これらの代りに硫化染料を使用することが研究され，市販の黒色硫化染料サルファブラックが防腐効果のあることが右田等[1]により報告されている。

著者等は農林産廃液である所の亜硫酸パルプ廃液及び硫酸ニコチン製造廃液を硫化して染料をつくり，綿糸を染着し，海水浸漬試験を行う事によつて防腐効果を検した結果を報告する。

実　験　の　部

漁網防腐試験の方法：農林産廃液を硫化する等の処理を行い，これを予め白煮した20番手5号綿糸に染着し，7月下旬より9月上旬迄，館山に於て海水浸漬を行い，10日目毎に抗張力の減少を測定してその防腐効果を検した。

防腐染料の調製と染着：

(1) 亜硫酸パルプ廃液（王子製紙会社苫小牧工場のパルプ廃液pH5，比重1.071）中に綿糸を入れ1時間煮沸後とり出して乾燥，重クロム酸カリ1%溶液中に30分浸漬固定，水洗1時間して日乾，この操作を5回繰返して染着を終つた。

(2) 同上パルプ廃液800g，コールタール200gを混じ，之に珪酸ソーダ50gを加えたものを50～80°Cで処理し，pH7.2のものを得た。之で綿糸を30分間60°Cで染着し室温で乾燥した。

(3) 同上パルプ廃液に50%炭酸ソーダを液量の7割加え，その上澄液pH9.6，比重1.061のものを1 l に対し，硫化ソーダ1.5kg，硫黄350gを加え130～150°Cに熱して硫化を行い，これを濃縮，1.2kgの黒色硫化染料を得た。この黒色染料を硫化染料染着法に従つて，綿糸に対して硫化ソーダ15%，炭酸ソーダ5%，食塩50%よりなる助剤とよく混和し，綿糸を浸し1時間煮熟して染着け，直ちに水洗1時間後日乾した。この日乾の終つた綿糸は醋酸ソーダによる後処理[2]を行つた。

(4) 硫酸ニコチン製造廃液（東洋高圧工業豊沼工場産pH9.6，比重1.087）2*l*に対して硫化ソーダ700g，硫黄700gを加え，130～150°Cで硫化，濃縮して1.6kgの褐色硫化染料を得た。この褐色染料を(3)に述べたと同様に綿糸に染着終了後重クロム酸カリによる後処理[2]を行つた。

海水浸漬試験結果：前述の染着綿糸4種と対照として素糸，カツチ染(カツチ2%溶液90°C 45分処理後，硫酸銅3%，重クロム酸カリ1%温溶液固定)，コールタール染（コールタール30分処理）計7種について海水浸漬による抗張力の減少を10日目毎に測定した結果がFig. 1である。

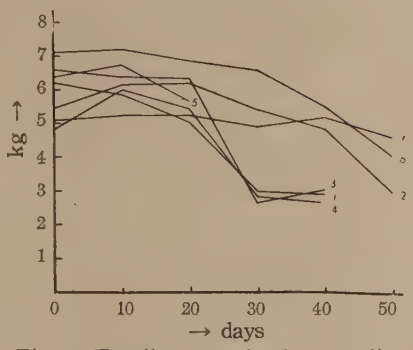


Fig. 1. Tensile strength of cotton lines exposed in sea water

1. Sulphite pulp waste liquor
2. Sulphite pulp waste liquor mixed coal tar
3. Sulphide dyes (Sulphite pulp waste liquor)
4. Sulphide dyes (Nicotine sulphate waste liquor)
5. Untreated line, control
6. Cutch 2% ($K_2Cr_2O_7.CuSO_4$), control
7. Coal tar, control

考察及び総括

亜硫酸パルプ廃液自体の防腐効果を検する為パルプ廃液のみの処理綿糸(1)について試験した結果は素糸より20日間切断が延びたのみであつた。(3),(4)は亜硫酸パルプ及び硫酸ニコチン製造廃液をそれぞれ硫化処理して染着したものであるが，両者とも素糸より20日間切断が延びたのみで特に効果の著しいものが無く，カツチ染及びコールタール染の対照に比して劣つた。(2)はパルプ廃液とコールタール，硅酸ソーダを混合処理したものであるが，対照の単なるコールタール染着に比してやゝ劣るが可成りの防腐効果を示した。このものはコールタールに比して染着後の乾燥も早く，しかも染着剤のベタツキも少くて取扱いが容易であると云う利点がある。之はパルプ廃液中のリグニンスルホン酸等の成分が染着の際滲透剤として有効に働いた為であろう。尚硫化染料で処理した(3)，(4)の綿糸に対する染着量を測定した所，その値が非常に低い値を示した事より防腐効果の劣つた一因がこの為であつた事を推定した。[3][4]

本研究は旧北大農学部水産学科に於て文部省科研費交付総合研究「漁網の研究」の分担研究として行つた。海水浸漬試験を担当された東京水大岡田教授研究室の諸氏並に試料入手斡旋の労をとられた北大農学部林産学科半沢助教授に深謝の意を表する。

文　　献

1) 右田・花岡 (1941). 水試報 (11).
2) 小川 (1937). 最新化学工業大系 **6**. (p.373).
3) 右田・花岡 (1942). 水試報 (12).
4) 右田 (1942). 日水誌 **11**.

農林産廃液の漁網防腐剤への利用

第2報　亜硫酸パルプ廃液の硝化後硫化処理試験

五十嵐久尙・田ノ上家久
（北海道大学水産学部水産化学教室）

Utilization of Waste Liquors of Agricultural and Forest Products for Net Preservatives

II. Experiment on the sulphuration of the nitration products of sulphite pulp waste liquor

Hisanao IGARASHI and Iehisa TANOUE

Abstract

Net preservatives which were made by sulphuration of the nitration products of sulphite pulp waste liquor, were tested by exposing in sea water cotton lines which had been treated with them. The dye to which copper sulphate had been added at the sulphuration showed remarkably increased preservative power. The use of surface active agents for dyeing had no influence on the preservation of the net.

著者等は前報[1]に於て亜硫酸パルプ廃液を単に硫化処理して漁網防腐剤を製したが効果が少いので，予め硝化を行い，更に硫化処理を行つた。防腐力を増強する為に硫化の際硫酸銅を添加したり，染着の際綿糸に対する滲透性を増す為に界面活性剤を使用して，海水浸漬によつてその効果を検した結果を述べる。

実　験　の　部

漁網防腐試験の方法：亜硫酸パルプ廃液を硝化後更に硫化して硫化染料をつくり，之を予め白煮した20番手5号綿糸に染着し，7月上旬より9月下旬迄館山に於て海水浸漬を行い，10日目毎に抗張力の減少を測定した。

防腐染料の調製と染着：

(1) 亜硫酸パルプ廃液は王子製紙会社苫小牧工場産 pH3.4，比重1.064 のものを減圧で乾固（比重1.250）に濃縮して用いた。濃厚廃液 500gを攪拌しつゝ 55～66°Cに於て32%硝酸 250ccを滴下反応させて硝化を行い，[2] 之を暗赤色粘調になる迄濃縮(全量を500gとする)，50%苛性ソーダで中和し，之を硫化ソーダ500g，硫黄250gで還流冷却下 130～150°C に熱して硫化を行い硫化染料を得た。次に第1報同様綿糸に対して，硫化ソーダ15%，炭酸ソーダ5%，食塩50%の助剤と共に加熱染着，乾燥後1%重クロム酸カリで固定した。

(2) 同上パルプ廃液を (1) 同様硝化，硫化処理によつて得た染料を以て綿糸に染着する際に，助剤にアニオン活性剤センデール（第一工業製薬）0.5%を加え前同様染着を行い，乾燥後固定液にカチオン活性剤カチオーゲンH(同上)を0.2%加えて固定した。[3][4]

(3) パルプ廃液を (1) 同様硝化後硫化を行う際に硫酸銅50gを加えて処理し染料を製し，(1) 同様染着固定した。

(4) (3)の染料を染着固定する際に(2)同様助剤にアニオン活性剤を加えて行い，且カチオン活性剤で固定した。

海水浸漬試験結果：上述の4種の染着綿糸と対照として素糸，カツチ染（カツチ2％溶液90°C 45分処理後，硫酸銅3％，重クロム酸カリ1％温溶液で固定）及びコールタール染（コールタール30分処理）計7種について海水浸漬による抗張力の減少を10日目毎に測定した結果はFig. 1である。

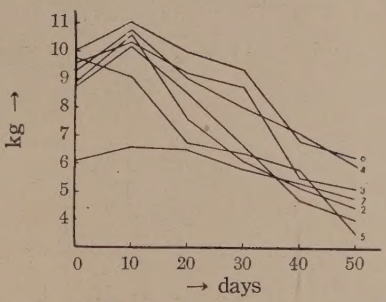


Fig. 1. Tensile strength of cotton lines exposed in sea water

Sulphide dyes (Sulphite pulp waste liquor)
1. sulphide dye only
2. treated with surface active agents
3. with $CuSO_4$ added
4. $CuSO_4$ added also treated with surface active agents

Control
5. untreated line
6. Cutch 2% ($K_2Cr_2O_7$. $CuSO_4$)
7. Coal tar

考察及び総括

亜硫酸パルプ廃液を硝化硫化した染料（1），(2)は素糸(5)に比し相当の防腐効果があつた。殊に硝化後硫酸銅を添加して硫化処理した染料（3），(4)は銅無添加のものに比して防腐効果が著しく増大した事を認めた。併し，染着及び固定の際界面活性剤を使用する事は特に防腐効果を増加させなかつた。

本研究は旧北大農学部水産学科に於て，文部省科研費交付総合研究「漁網の研究」の分担研究として行つた。海水浸漬試験を担当された東京水大岡田教授研究室の諸氏並に試料入手斡旋の労をとられた北大農学部林産学科半沢助教授に深謝の意を表する。

文　　献

1) 五十嵐・田ノ上（1955）. 北大水産彙報 **6** (1), 80.
2) Doree, C.（1924）. *J. Soc. Chem. Ind.* **43**, 261T.
3) 山本（1952）. 界面活性剤とその応用.（p.193）.
4) 小竹・赤堀（1946）. 有機化学の進歩. 第4輯.（p.125）.

昭和30年 5 月 1 日印刷
昭和30年 5 月15日発行

編集人 元 田 茂
函館市港町253 北海道大学水産学部

印刷所 第 一 印 刷
函館市恵比須町1の2

印刷人 板 垣 雄 二
函館市恵比須町1の2

発行所 北海道大学水産学部
函館市港町253

BULLETIN
OF THE
FACULTY OF FISHERIES

Vol. 6, No. 1

CONTENTS

IGARASHI, Hisanao & TANOUE, Iehisa: Utilization of waste liquors of agricultural and forest products for net preservatives. I. Experiment on the sulphuration of waste liquors of sulphite pulp and nicotine sulphate 80

———— & ————: Utilization of waste liquors of agricultural and forest products for net preservatives. II. Experiment on the sulphuration of the nitration products of sulphite pulp waste liquor 82

KATO, Kenji: Regional distribution of organic matter in bottom sediments in the vicinity of Okushiri Strait, Hokkaido 19

KOBAYASHI, Shinjiro: Changes in catalase activity of the tissues and blood of "Masu", *Oncorhynchus masou*, when transferred from fresh-water to sea water 1

MOTODA, Sigeru: Vertical distribution of mackerel eggs in Ishikari Bay, Hokkaido 7

———— & ANRAKU, Masateru: Occurrence of mackerel eggs in Ishikari Bay, Hokkaido, in June, 1954, as investigated by underway plankton catcher 12

———— & ————: Further observation on the daily change in amount of catches of plankton animals in vertical hauls 15

TAKAGI, Mitsuzo & MURATA, Kiichi: Studies on the mechanism of nitrogen assimilation in marine algae-VI. The variation of nitrate reductase activity according to the portions and to the frond length of some marine algae 25

———— & ————: Studies on the mechanism of nitrogen assimilation in marine algae-VII. Effect of various inhibitors on nitrate reductase 29

———— & ————: Studies on the mechanism of nitrogen assimilation in marine algae-VIII. On the electron carrier of nitrate reductase in *Porphyra yezoensis* 33

TANIKAWA, Eiichi, AKIBA, Minoru, ISHIKO, Hirotoshi & YOSHITANI, Setsuko: Studies on post-mortem changes in the chemical constitution of the meat of sea cucumber (*Stichopus japonicus SELENKA*). I. Changes in the amounts of lactic acid and glycogen in the meat of *Stichopus japonicus* during the period of rigor mortis 52

————, ———— & YOSHITANI, Setsuko: Studies on post-mortem changes in the chemical constitution of the meat of sea cucumber (*Stichopus japonicus SELENKA*). II. Autolysis of the meat of *Stichopus japonicus* 57

———— & ISHIKO, Hirotoshi: Studies on the nutritive value of the meat of sea cucumber (*Stichopus japonicus SELENKA*). V. Nitrogen distribution and amino acid composition of protein of meat of *Stichopus japonicus* 37

————, ———— & MOTOHIRO, Terushige: Studies on the nutritive value of the meat of sea cucumber (*Stichopus japonicus SELENKA*). VI. Nitrogen distribution and kind of chemical components of the extractives of the meat and skin parts of *Stichopus japonicus* 42

————, MOTOHIRO, Terushige & WAKASA, Tetsuro: Studies on post-mortem changes in the chemical constitution of the meat of sea cucumber (*Stichopus japonicus SELENKA*). III. The putrefaction of the meat of *Stichopus japonicus* 63

————, ———— & ————: Studies on post-mortem changes in the chemical constitution of the meat of sea cucumber (*Stichopus japonicus SELENKA*). IV. Determination of the freshness of the meat of *Stichopus japonicus* and the limit of the freshness for use as the raw material of canned food 73

———— & WAKASA, Tetsuro: Studies on the nutritive value of the meat of sea cucumber (*Stichopus japonicus SELENKA*). VII. Inorganic matter in the meat of *Stichopus japonicus* 49